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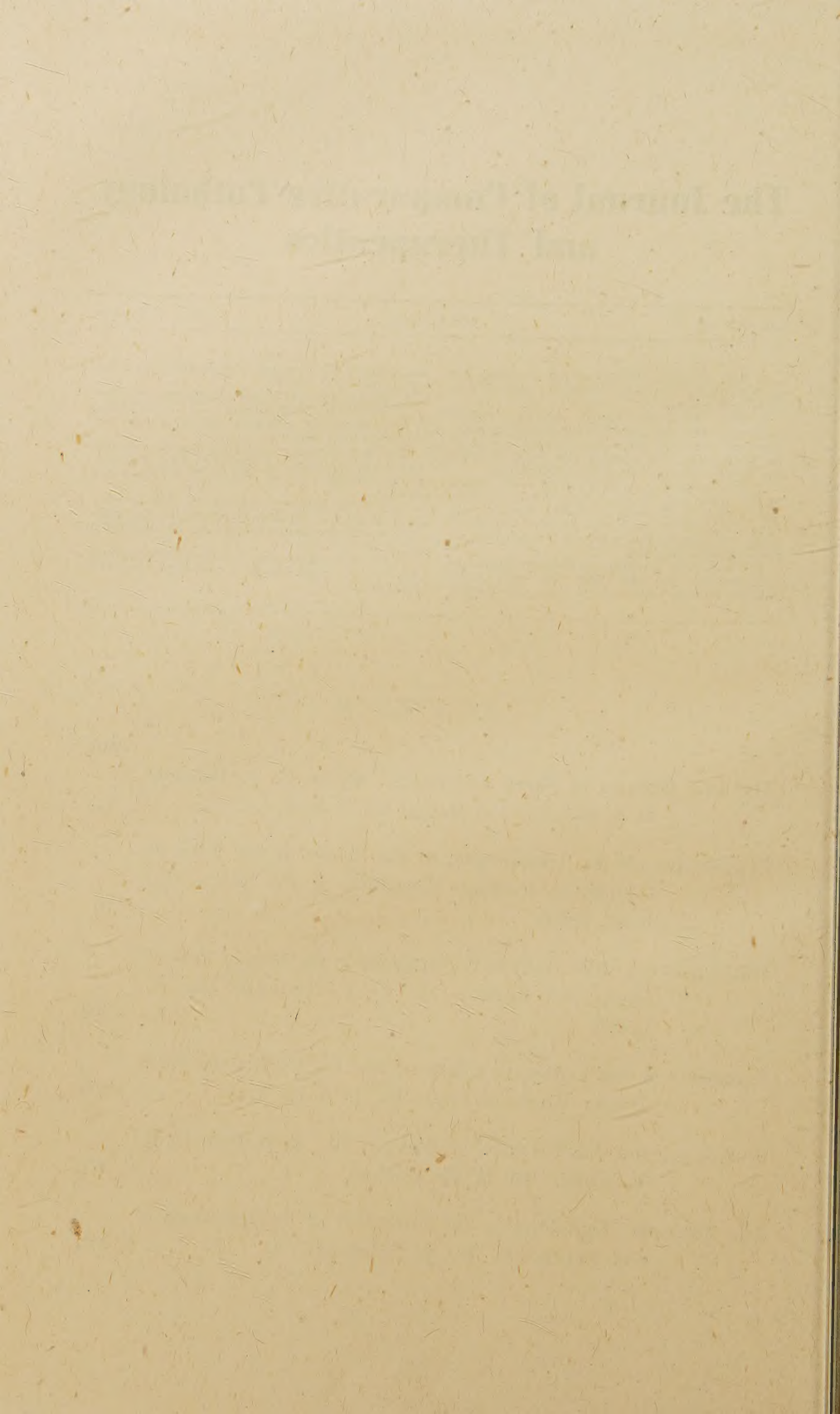
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THE POX DISEASES OF SHEEP AND GOATS

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INTRODUCTION

It is not proposed to give a detailed review of this subject. Bridré (1931) and Glover (1931), in their summaries, concluded that there was a fair measure of agreement on the status of the poxes. On a few points, notably the interrelationship of goat-pox and contagious pustular dermatitis, they found, however, that little or no sound information was available. Furthermore, opinions conflicted on the relationship of vaccinia to the other poxes. On this subject a minority, comprising the "unicists," differed from the "pluralist" majority in regarding vaccinia as a kind of basic pox to which all others were related. The unicist theme seems roughly to be that any pox, by passing in certain ways through certain animals, can be transmuted into a virus immunologically identical with vaccinia, while vaccinia, on passage through the natural hosts of the other poxes, can be transmuted into the naturally occurring poxes of these animals. Doubtless the proved interrelationship of the viruses constituting the variola-alastrim-vaccinia group is responsible for this hypothesis. Work already carried out by two of us (E. S. H. and M. A. H.) on human pox infections had shown that a good deal of discrepancy in the interpretation of experimental results could be eliminated by an improved technique in preparing and inserting the virus used in critical infection tests.

The present work arose out of the discovery of sheep-pox in the Sudan, a disease which has probably always existed in the country, although it had not come to veterinary notice. The circumstances in which it was ultimately discovered were such that it was not certain that a large-scale epizootic was not impending, and so it seemed that widespread immunisation might possibly be required. Although it was thought likely that "clavelisation" would be the only immunisation method of any use, there still existed three uncontradicted claims that safer and equally effective methods were available. These were: (1) that vaccinia alone would serve, provided it was of high potency; (2) that vaccinia could be "ovinised," or for all practical purposes transmuted into a mild kind of sheep-pox virus, and would provide a safe and effective vaccine; and (3) that the sheep-pox virus could be attenuated by the process of "caprinisation," or passage through goats, and would then serve as a safe, effective vaccine. Since smallpox vaccine is prepared locally in the Stack Laboratories, and since sheep and goats are cheap and plentiful in the Sudan, it was decided to check these claims using the improved technique already mentioned. As will later be shown, they could not be confirmed.

Work on goat-pox was undertaken mainly because so little was known about this disease, which was not known to occur in the Sudan. As will become evident at a later stage, it can now be confidently said that in fact it does not occur. On the other hand, it was known to be prevalent in Palestine where sheep-pox does not exist, although the latter is quite common in the contiguous territory of Egypt. It was not, of course, thought on this account that there was necessarily any close immunological relationship between sheep-pox and goat-pox, but the relationship of these two poxes to each other and of goat-pox to vaccinia seemed well worth studying by means of our technique. Studies on contagious pustular dermatitis almost automatically followed because of the uncertainty regarding its relationship to goat-pox.

The work now to be recorded thus falls into six sections, namely: (1) the technique employed; (2) brief notes on sheep-pox in the Sudan, and the relationship between (3) sheep-pox and vaccinia (including "caprinised" sheep-pox and "ovinised" vaccinia), (4) goat-pox and vaccinia, (5) sheep-pox and goat-pox, and (6) contagious pustular dermatitis and goat-pox.

EXPERIMENTAL TECHNIQUE

Stress is mainly laid on the factor of technique, to eliminate imperfections which are concerned both with the actual infective material and with the manner of its insertion.

Material Used

The experience of the writers confirms them in their view that dried scabs currently used as the infecting material in experiments on the animal poxes are likely to produce equivocal results. When freshly gathered, such material contains numerous bacteria and is apt to produce impetigo when inserted by scarification. Conversely, after it has been stored long enough for most of the bacteria to disappear, the titre of the virus, already probably low—as it usually is in scabs as compared with vesicles—becomes appreciably lower. The most equivocal results are apt to be obtained when the material has been stored for intermediate periods, when a combined bacterial and virus infection arises that is often difficult to assess. With sheep-pox, where the true pox lesion is large and persistent, and usually haemorrhagic, the danger of confusion is negligible, but with goat-pox, which has smaller and more transient lesions, confusion, in the experience of the writers, is quite possible. This limitation would apply *a fortiori* to contagious pustular dermatitis, with its still smaller and still more transient lesions.* The writers cannot claim, however, to have tested their own powers of assessment in this case, but they suggest that the divergent results obtained

* See, however, subsequent observations on the character of contagious pustular dermatitis lesions in monkeys.

by other workers in the past may have arisen from combined pox and bacterial eruptions.

Three techniques were successively studied. Realising the tendency to a low virus titre in scabs, the aim always was (1) to take material from infected animals at the peak of local reaction, (2) to eliminate pyogenic bacteria, and (3) to titrate the bacteria-free product immediately, while it was still in the freshest possible condition. Comparative titrations were actually carried out on sheep-pox material only. The findings were found, as was proved subsequently, to apply to the other poxes. The three infective preparations comprised (i) etherised elementary bodies, (ii) etherised papule pulp, and (iii) etherised vesicle contents, prepared as follows:—

Elementary Bodies ("E.B.s").—These were selected for the first titration because a fully active E.B. suspension must evidently be of a titre unsurpassable by any other preparation. Levaditi, Bridré and Krassnoff (1938) found the E.B.s of sheep-pox to be slightly larger than those of vaccinia: hence suspensions of the former ought to be obtainable by methods suitable for concentrating the E.B.s of vaccinia. These E.B.s could doubtless have been prepared from any infective material, from early papule to late scab, as all debris and adventitious micro-organisms would be removed in the process of preparation. They were actually prepared, however, from the large sheep-pox plaques, about 3 cm. in diameter and nearly 1 cm. high, developing after intradermal injection. The plaques, which reached their maximum size on the seventh or eighth day, were removed surgically from the living sheep under local anaesthetic, and were treated by a shortened modification of the technique of Macfarlane and Salaman (1938) whose whole process of purification was not considered necessary because the same objective was not in view.

The tissue was chopped up finely with scissors, ground in a mortar with Pyrex glass powder, and M/250 McIlvaine's buffer (citric acid-disodium phosphate) at pH 7.0 slowly added during grinding until 10 c.c. had been added for each 1 g. The E.B.s were recovered by two washings in the buffer solution, each consisting of first throwing down debris by half an hour's spinning in an ordinary laboratory centrifuge, and then throwing down the E.B.s from the supernatant fluid by prolonged centrifugation (two and a half hours) in a Lundgren high-speed angle-centrifuge. The high-speed centrifuging was always carried out inside a refrigerator, since it was feared that exposure to room temperature (100° to 110° F.) for so long a period might be harmful. The deposit of nearly pure E.B.s recovered after the second washing was suspended in a volume of buffer solution five times the bulk, w/v, of the tissue originally excised, the suspensions so prepared having about the opacity of milk. The sheep employed afterwards all made uneventful recoveries.

Papule Pulp.—Papular pulp is used to denote that the primary material was taken from infected sheep showing the relatively early stage of the lesion as distinguished from those in the vesicular or vesicular-pustular stage from which the material dealt with under the next heading was gathered. Actually, however, the material consisted of excised plaques such as have been already described, since although they are very large these are essentially also lesions of the papular stage. The inoculum was prepared by excising, chopping,

and grinding with glass powder in buffer solution as above, but, in order to have a comparable dilution of infective material, only five times the volume of buffer was added. Coarse débris was removed by spinning in an ordinary centrifuge for half an hour, the deposit being discarded.

Vesicle Contents.—This material was gathered at a later stage of the eruption, either from naturally affected sheep or those artificially infected by scarification. It was ground with five times its volume of buffer solution and stored without further treatment in a refrigerator. Immediately before use it was centrifuged for a short time to remove coarse particles.

Note on Etherisation

Washed E.B.s contain very few bacteria, no matter what material is used for their preparation. Papule pulp, similarly, will contain hardly any micro-organisms except a few of extraneous origin which gain access during excision and subsequent manipulation. Vesicle or vesico-pustular material, however, is always heavily contaminated. All infective pox material used in these experiments was therefore treated with ether immediately before injection into or scarification on to the animals. This statement applies to all the diseases considered in this paper. The routine method of the Stack Laboratories for reducing the bacterial content of small-pox vaccine was used, 10 per cent. of ether being added to the vaccine "lymph," and the mixture then well shaken in a stoppered bottle, which is stored in a refrigerator for eleven to twelve hours. The ether is removed by bubbling filtered air through the mixture until no smell of ether remains; this takes about ten to 15 minutes for 40 to 50 c.c. of suspension. Cultural tests on the material then show that all pyogenic organisms which might obscure a pure pox reaction have been eliminated, and no bacteria remain alive except a few bacilli of the *B. subtilis* type which presumably survive the treatment in spore form. There are also, as a rule, a few corynebacteria, but these seem to be non-pyogenic.

Note on Volume and Dilutions

All suspensions of infective material were made up to five times the volume of the original tissue or vesico-pustular material recovered from the virus-producing animals. The suspension in 1/5 concentration, the basic material from which dilutions are made for titration of potency, is for convenience termed "undiluted virus" and designated 1/1. Dilutions of 1/10, 1/100, etc., are made from it.

As was to be expected from the results of cultural tests, titration of the above three preparations on sheep showed that in no case did bacterial lesions develop either on scarification or intradermal injection. Fortunately, also, no difference in titre could be detected provided site and route of infection were constant. The one selected for use in critical experiments was therefore the third, *i.e.*, etherised vesicle contents, which was the easiest to prepare. Furthermore, it was the only one of the three that could be prepared with any degree of certainty in the case of goat-pox and contagious pustular dermatitis. As the size of their virus particles is not known the assurance was lacking that washed E.B.s could be secured in adequate quantity. Moreover, as the papules produced by intradermal injection are very small it would have been very difficult to produce enough papule pulp. No attempt was made, therefore, to test the other two kinds of preparation in these two instances: all that was done was to ascertain whether etherised vesicle

material had a constantly high titre, which as already said it fortunately proved to have.

"Vesicle contents" may not be a good term to use for describing material gathered from an eruption in which vesicles rarely develop, as they rarely do in Sudan goats suffering from contagious pustular dermatitis. There was, however, no difficulty in securing corresponding material by gently scraping the surface of skin papules on the fifth day. The epithelium then easily became detached, in contrast to the tenacity with which it held fast later on. Contagious pustular dermatitis never produces large well-filled vesicles. It is probable that the tendency to vesiculation is specially weak in the Sudan because of the intensely hot, dry atmosphere. Desiccation presumably proceeds *pari passu* with exudation. At any rate, fifth day scrapings behaved in an entirely satisfactory manner.

Technique of Insertion

In order to avoid faults of earlier workers such as small number of animals used, age of infective material, route of infection, site of infection, area of skin scarified, and interval elapsing in cross-immunity experiments between immunising with one virus and testing with another, the following precautions were taken:—

Number of Animals in Experiments

With sheep-pox this is important for two reasons, one of which probably only applies to countries where the disease is enzoötic, while the other relates to the route of infection. In the Sudan, where sheep-pox is enzoötic, not more than about two-thirds of adult sheep are susceptible. Probably about 10 per cent. of yearlings are resistant. Whether this is due to the influence of age or to the greater chances of older sheep having recovered from natural infection is not known. On one occasion, during preliminary experiments in which only one or two sheep were used, the writers thought that their virus had lost its potency, because, after a series of successful infections, four sheep in succession proved resistant. No conclusion, therefore, was subsequently considered valid unless deduced from the behaviour of at least half a dozen sheep. These remarks apply to sheep injected intradermally. The proportion found resistant to infection by scarification would undoubtedly have been larger (see following notes on route of infection). With the other pox viruses the number of animals used is not so important, since the diseases caused by them do not occur in the Sudan and not one of the hosts naturally susceptible has yet been found immune. Nevertheless, several animals were always used in an experiment. This precaution has not always been observed in countries where the diseases are enzoötic and there is a good chance that many of the natural hosts employed were partially or wholly immune.

Age of Infective Material

All doubts regarding the viability or maintenance of potency have been avoided in our experiments by using the freshest possible material. For all critical cross-immunity tests a special batch was always prepared, and used immediately it was ready.

Route of Infection

Scarification is the orthodox mode of infection in pox experiments. In the case of sheep-pox, it is suggested by some workers that intradermal

injection is more certain. Gins and Kunert (1937) practised insufflation into the nostril. Scarification and intradermal injections were therefore compared using the three sheep-pox preparations already described. Intranasal insufflation was not investigated because a study of Gins and Kunert's paper indicated it to be so uncertain that it was obviously not worth considering. In parallel tests with the three preparations (25 sheep used altogether at various times), the more certain route was found to be intradermal injection. Serial dilutions, ranging from 1/1 to 1/10,000 were used. As already recorded all three preparations behaved in an identical manner. No sheep injected intradermally developed lesions without showing them in the 1/10,000 site. But several sheep developing lesions in the low-dilution sites after scarification failed to develop them in the high-dilution sites. One sheep failed to develop any scarification lesions whatever, even in the 1/1 site, while it reacted uniformly to all intradermal injections from 1/1 to 1/10,000. Unless Sudan experience is exceptional, it is thus evident that conclusions drawn from sheep-pox infection experiments must be heavily discounted if infection is effected by scarification. All attempts to infect with sheep-pox animals other than the sheep by both methods completely failed. The other pox viruses studied infected all test animals in the highest dilutions tried, by scarification, and so there was obviously no need to seek for any alternative.

Site of Infection.

Again it was only with the sheep-pox virus that the site proved highly significant: moreover, since intradermal injection invariably proved effective in the highest dilutions, it was only significant when infection was attempted by scarification. Of the four sites tested by scarification, the suitability, in order named, was: under surface of tail, wall of abdomen, inside of thigh, and skin of muzzle. Intradermal injection, however, is obviously the only route that should be used in critical experiments.

With the other three viruses good lesions invariably developed on the skin of the abdomen not only in sheep, but also in all other animals tested, and there was no point in looking further.

Area of Skin Scarified

As others have pointed out, the degree of immunity produced may be significantly influenced by the area of skin scarified. Horgan and Mansur (1939) summarising the literature in so far as human pox diseases are concerned, show that in monkeys neither variola nor alastrim protects fully against vaccinia unless a large quantity of virus, producing an extensive local reaction, is used in the preliminary immunisation. The same workers (publication in press) have further shown that this also applies to human beings vaccinated with vaccinia. In a large number of adults revaccinated and therefore possessing varying degrees of partial immunity from an earlier vaccination, it was found that when revaccination was performed by two linear insertions the proportion of "takes" (vaccinoid reactions) was larger than when a single insertion was made. Therefore, if revaccination had been carried out by one insertion, many more individuals would have been recorded as "negative" (immediate reactions). Whether a quantitative factor operates in the case of the pox diseases of sheep and goats is not known. If it does it may explain some of the divergence of opinion that exists in regard to the immunological status of these diseases. The writers took good care in the present experiments that inadequate dosage should not vitiate their results. Every animal scarified, both in the process of immunisation

and in immunity tests, was scarified over an area of several square inches. In the case of sheep-pox multiple large doses of concentrated virus were injected intradermally.

Interval Between Immunisation and Test

This is not always recorded in publications. As immunity must wane, however slowly, after having reached its peak, cross-immunity tests should be carried out fairly soon after preliminary immunisation. Since the maximum immunity to vaccinia is known to develop in well under a month, it is presumed that it will do so after much the same interval with the other poxes. It would not, as a rule, have been possible to test immunity in the present experiment at an interval of much less than a month, because virtually the whole of the posterior abdominal surface was scarified with the first virus (except in the case of sheep-pox). Time had, therefore, to be allowed for the last lesion to heal on the animal showing the most persistent local reaction before inserting the second virus. Although the interval will be recorded in each experiment, it is not superfluous to state that cross-immunity tests were, so far as was practicable, carried out at an interval of *about* one month.

Premises

All due precautions were taken to prevent accidental intrusion of an unwanted virus through using possibly infected premises. Vaccinia is well known to be a great menace in this respect, and, since small-pox vaccine is prepared in the Stack Laboratories, work with viruses other than vaccinia was done in the Veterinary Research Laboratories, of which the nearest group of buildings is about a mile distant and includes two quite separate stock yards, and a second group is situated yet another mile distant on the far side of the Blue Nile.

Arising out of the foregoing, the following technique was finally adopted: The virus used, whether in susceptibility tests, initial immunisation, or cross-immunity tests, was, unless otherwise stated, fresh etherised vesico-pustular material. In the case of sheep-pox it was always injected intradermally in the skin under the tail,* and in the case of the other viruses it was scarified on to the skin of the lower abdomen. Several animals were always used, all of which were scarified over several square inches of skin so as to insert the largest quantity practicable of virus and produce the maximum reaction possible. In initial immunisation only 1/1 material was inserted, but in cross-immunity tests a range of dilutions was sometimes used. Cross-immunity tests were, moreover, always carried out at the shortest interval after initial immunisation.

SHEEP-POX IN THE SUDAN

Since the presence of this disease was not definitely established in the Sudan until 1940, a few remarks on it may not be superfluous.

* Actually the tail did not show itself superior to the belly where intradermal injection was at issue. However, it did so when tested by scarification and was thus regarded as the most susceptible site. Incidentally, since all local sheep are of what Lydekker (1912) terms the "long tailed" type, having long tapering tails with a broad, thin-skinned, hairless area extending nearly half-way down the under surface, this surface was ideal for observation.

Incidence in Local Sheep.—From enquiry among native sheep owners and dealers the disease is quite well known, but is said to have a slow rate of spread and to cause few deaths: severe cases in which the prospect of recovery is doubtful are usually slaughtered and eaten. These statements concerning the reputed natural occurrence of the disease would explain why the disease has not hitherto come to veterinary notice.

Observations on densely crowded sheep, estimated at hundreds of thousands, and experiments in the laboratory, have confirmed the above view. As soon as the identity of the disease was established, a special look out was kept for cases occurring in the Khartoum and Wadi Halfa export quarantines. Among nearly 140,000 sheep specially examined consecutively over a period of several months, only 56 cases of sheep-pox were detected. The sick sheep were taken out for local slaughter, and among the remainder, which continued their journey to Egypt, only two further cases were reported as being detected in Egyptian abattoirs. Even if these figures are not quite accurate they confirm the native contention that the disease has a slow rate of spread. Although adult sheep, which are the only ones exported, are more resistant to infection than younger sheep, the slow rate of spread is not entirely referable to immunity. Of 31 sheep tested in the laboratory the susceptibility was as follows:—

		<i>Tested.</i>	<i>Lesions.</i>	<i>Fever only.</i>	<i>Immune.</i>
Adults	...	24	14	2	8
Yearlings	...	7	6	1	Nil

According to native sheep owners, natural sheep-pox shows a strong tendency towards generalisation. Generalised cases have certainly been detected in the quarantines, but in non-generalised cases the commonest site in which to find a few lesions has been under the tail. A significant admission on the part of sheep dealers questioned is that if they see lesions simulating pox on the head and body they usually look under the tail to confirm their diagnosis.

Further enquiry reveals that British veterinary officers in the past have been shown tail lesions of reputed sheep-pox which they have dismissed as tick sores. In the writers' opinion these were probably true pox lesions. The large papule described as a "tick sore" in horses is invariably caused by ticks of the genus *Amblyomma*, which is a genus found in latitudes more southerly than those in which sheep of export type are raised. Furthermore, the two local species of this genus, *A. lepidum* and *A. variegatum*, have never been found on sheep in the writers' experience, and sheep are not included in the list of hosts recorded by Robinson (1926). Incidentally it seems also that the common tick of the northern sheep areas, *Hyalomma aegyptium*, does not attach itself under the tail of sheep, although this is its favourite habitat in horses and cattle. Sheep tied up in a yard badly infested with these

ticks were viciously attacked inside the ears but nowhere else. These observations are of little importance except that they afford corroborative evidence on two points, namely, that sheep-pox is fairly widespread in the Sudan, although earlier literature does not record its existence, and that the most susceptible area of a sheep's skin is that under the tail.

Severity in Local Sheep.—Although incidence is low and rate of spread slow, there is no doubt that sheep-pox is a serious disease for such sheep as become infected. This might have been deduced *a priori* from the information that pastorals, who are always very averse to slaughtering any animal, will eat sheep-pox sheep. More definite evidence, however, came to hand in the course of these experiments. Since the work was, in the first place, undertaken in circumstances in which exporting merchants' interests appeared to be at stake, sheep for experiment were borrowed from them on the understanding that they would be returned in good condition later on, payment being made only for those not acceptable. This arrangement had to be cancelled after the first returns were made, since although they had been only artificially infected and had developed only local lesions (usually with fever, however) and had subsequently been given a clear month's rest with good feeding, the sheep nearly all died on their journey to Egypt. Although "clavelisation" does not lead to generalised sheep-pox, this observation indicates that it is by no means a procedure to be lightly undertaken in sheep which will subsequently have to undergo hardship. It would certainly not have been a practicable method of immunisation for the export sheep in which it was originally thought that some kind of vaccination might become necessary.

Summary

Sheep-pox has probably existed in the Sudan for many years. Its rate of spread is very slow, and, although there is a tendency to generalisation, local lesions under the tail are probably the commonest manifestations of the disease. In spite of its negligible importance from an epizootiological standpoint, it can be a very serious affection for such sheep as become infected.

RELATION OF SHEEP-POX AND VACCINIA

Range of Pathogenicity.—The balance of evidence is overwhelming in the sense that, while vaccinia is easily transmissible to almost any mammal, sheep-pox is definitely not so transmissible, or can at most be transmitted in special circumstances that cannot regularly be reproduced. Since the field for investigation on this point is well-nigh limitless, the writers have no intention of exploring it. Bridré (loc. cit., 1931) quotes claims that sheep-pox has been transmitted to the horse, rat, guinea-pig, Dorcas gazelle, and Barbary sheep, and other claims may be found on consulting more recent

literature. The writers have merely failed to transmit it to the animals most commonly used in pox work, namely, rabbit, monkey,* and calf, but see no reason why an open mind on the claims of other workers should not be maintained.

Borrel and Konev (quoted by Bridré, loc. cit., but references not given and untraceable in locally available literature) and Kasai (1927) have claimed that sheep-pox is not only transmissible to goats but can be serially passed in them, until after 15 to 20 passages it is attenuated for sheep ("caprinised") and constitutes a safe and effective immunising agent. It seems, however, that these claims to have transmitted sheep-pox to goats have emanated from lymph institutes, where intrusion of vaccinia is notoriously possible, and the claims that "caprinised" virus is a good immunising agent against sheep-pox have been based on circumstantial evidence obtained in the field, and not proved by critical laboratory experiments.

Nevertheless the claims had such far-reaching potentialities that the writers decided to check them. Three separate attempts were made, with three, two, and three goats, respectively, to produce infection by multiple intradermal injections of 0.2 c.c. of a 1/1 suspension of sheep-pox E.B.s. Not one of the seven goats developed a pox lesion although the suspension infected control sheep at a 1/10,000 dilution. On one occasion some of the areas of the skin around the sites of infection were excised, treated as for concentration of E.B.s, and injected into two further goats. Neither of these developed a pox lesion. It seems, therefore, that goats resemble other animals in that they are at least not *ordinarily* susceptible to sheep-pox.

Immunological Relationship.—It is generally agreed that infection with any pox virus confers protection against reinfection with the same virus, and the balance of opinion is overwhelming that, outside the variola-alastrim-vaccinia group, no mammalian pox has been shown to protect against any other. (The behaviour of goat-pox and contagious pustular dermatitis, which calls for a modification of the second half of the above statement, will be dealt with later.) Nevertheless two claims have been made by Kii and Kasai (1927) and by Gins and Kunert (1937), that vaccinia will protect against sheep-pox. The claim of Kii and Kasai is complicated by a transmutation hypothesis, on the usual lines to the effect that vaccinia can be "ovinised" by passage through sheep. It is not, however, "ovinised" in the sense that the sheep-pox virus already mentioned was said to be "caprinised," i.e., merely attenuated while still remaining essentially the same pox. It becomes, they allege, actually transmuted into sheep-pox. In proof of this they claim that a vaccinia virus, originally infective and protective for children and

* The only monkey used throughout these experiments is the local grivet, *Cercopithecus aethiops*.

rabbits but non-protective against sheep-pox, becomes, with repeated sheep-passage, non-infective for rabbits and children, and protective against sheep-pox in sheep. The claim is, however, tempered by the admission that the immunity produced in sheep is too weak to be of any practical value, thus engendering the regret that the protocols, being in Japanese, cannot be examined in detail. Gins and Kunert, following up the earlier work of Gins (1920), confine themselves to intranasal insufflation and instillation of both the immunising and the test virus. After first maintaining that "ovinised" vaccinia immunises against sheep-pox (complete "ovinisation" being proved by the failure of the transmuted virus to infect one calf, which was not subsequently tested with normal vaccinia), they later claim to demonstrate that even unaltered vaccinia virus is equally as effective. Gins and Kunert, it must be concluded, have failed to make out any case, either for "ovinised" or for unaltered vaccinia. Nevertheless their work has sometimes been accepted at the face value of their conclusions, and it cannot, therefore, be ignored.

The writers were particularly well situated for checking both these claims in one operation, because the smallpox vaccine regularly prepared in the Stack Laboratories is virtually "ovinised" vaccinia. The virus is maintained for the most part in sheep, and all vaccine issued for field use is gathered from sheep. It is, incidentally, and in contrast to Kii and Kasai's claim, highly potent (1/100,000 to 1/500,000) for rabbits, and certainly does not fail to produce good "takes" in human beings. Actually, it must be admitted that continuous sheep-to-sheep passage is not practised as a routine, but every few generations the virus is passed through a monkey. This out-passage, however, has no immunological implications, and is merely done because experience has shown that the largest yield of pulp from each animal can be best attained by adopting the practice. The complete absence of change in the range of pathogenicity was, indeed, checked some years ago by carrying out ten consecutive E.B. sheep-passages, titrating the potency every two or three passages in rabbits. Not only did the original high titre for rabbits remain unaltered throughout the series, but lymph from the 10th passage, when issued for field use, produced quite as good "takes" in human beings as the standard routine lymph. However, using the routine local smallpox vaccine virus and the Sudan strain of sheep-pox virus, cross-immunity experiments were carried out on sheep, the details being as follows:—

1. *Does Vaccinia Protect Against Sheep-Pox?*

A small-scale test on three sheep recently recovered from vaccinia showed them to be immune to 1/1 vaccinia and as susceptible to sheep-pox as a single control, *i.e.*, up to 1/10,000, the highest dilution of virus used. The importance of the point at issue, how-

ever, justified the carrying out of a larger experiment. Twelve vaccinated sheep and six controls were used. The vaccinated sheep were scarified over the whole surface of the belly with 1/1 vaccinia suspension, and all developed extensive severe "takes" with high fever. After an interval of 29 days all these, together with six clean controls, were injected intradermally under the tail with 0.2 c.c. of 1/1 sheep-pox papule pulp suspension. The immunity to vaccinia was not checked, as such a check was considered unnecessary. The result is shown in Table I.

TABLE I
TEST OF IMMUNITY TO SHEEP-POX IN SHEEP INFECTED 29 DAYS EARLIER
WITH VACCINIA

	Sheep No.	Febrile Reaction	Local Reaction.*
Recovered vaccinia sheep	34	+	O
	35	O	+++
	36	O	+++
	37	++	+
	38	+	+++
	39	+	+++
	40	O	+++
	41	O	O
	42	++	O
	43	O	O
	44	+	+++
	45	O	O
Controls	52	+	+++
	53	++	++
	54	++	O
	55	+	+++
	56	O	+
	57	+	O

* In this, as in all other tables in this paper, the plus marks describing local pox reactions mean: +++ = confluent vesicles, ++ = semi-confluent vesicles, and + = discrete vesicles.

This table demonstrates that the sheep previously infected with vaccinia were no more immune to sheep-pox than fresh sheep. It also demonstrates the need for using several sheep where sheep-pox is used as the test virus.

2. Does Sheep-Pox Protect Against Vaccinia?

It seemed *a priori* that this question would be answered in the negative. Of hundreds of sheep used in recent years for the preparation of smallpox vaccine not one had ever proved refractory to vaccinia, although some of them must have been recovered sheep-pox cases. They may not, however, have been *recently* recovered cases, although it has never been put forward that sheep-pox immunity disappears in the span of the average sheep's lifetime. After

a preliminary test on two sheep recently recovered from natural sheep-pox and two similarly recovered from artificial infection had shown them to be susceptible to vaccinia, a controlled experiment was carried out using half a dozen accumulated recovered sheep and three controls. This experiment was smaller than that designed to test cross-immunity in the reverse direction because no such sheep had ever been found immune to vaccinia. The three controls were not observed throughout the experiment but were scraped at an early stage for smallpox vaccine production. The result of the experiment is shown in Table II.

TABLE II
TEST OF IMMUNITY TO VACCINIA IN SHEEP RECOVERED FROM NATURAL
AND ARTIFICIAL SHEEP-POX

	<i>Sheep No.</i>	<i>Interval</i>	<i>Febrile Reaction</i>	<i>Local Reaction</i>
Recovered sheep-pox cases	1 *	61 days	++	+++
	2	60 "	++	++
	3 *	48 "	+	+
	5	44 "	+	+
	6	34 "	+++	+++
	7	34 "	+++	+++
Controls	A	—	Not observed	All good "takes" but scraped early for vac- cine production.
	B	—		
	C	—		

* Nos. 1 and 3 were natural cases showing generalisation. The remainder had been artificially infected with multiple intradermal injections of 1/1 papule pulp suspension.

Summary

The above experiments on vaccinia and sheep-pox fail to demonstrate any original relationship between the two viruses, and also fail to confirm that sheep-pox can be modified by "caprinisation" or that vaccinia can be modified by "ovinisation."

RELATION BETWEEN GOAT-POX AND VACCINIA

As little was known about goat-pox, work was undertaken upon a virus (dry scab) obtained from Mr. H. R. Binns, Senior Veterinary Research Officer, Palestine, whose readiness to co-operate and provide information is hereby gratefully acknowledged. Goat-pox is widespread both in sheep and goats in Palestine, but sheep-pox does not exist there; nor, so far as is known, does contagious pustular dermatitis. Goat-pox does not occur in the Sudan, and so the conditions for work, using our new technique, were highly favourable. It was thus proved beyond doubt, using the Palestine goat-pox material, that a purified virus must be used if pox cross-immunity experiments are to give clear-cut results. This holds true especially

with the viruses that give relatively early and transient reactions and are as a rule unaccompanied by fever.

Two goats, scarified on the abdomen, thighs and lips with some of the original material suspended in saline, developed marked impetigo commencing on the second day. Skin lesions persisted until the 12th and 19th day respectively. The writers, however, were not prepared to say at any stage in the progress of the infection that they were dealing with an uncomplicated pox eruption. All goats infected subsequently were scarified, on the abdomen, with etherised vesicle contents, and a classical, uncomplicated pox picture developed in all of them. Observations on 23 goats in all showed that erythema and papule formation appeared on about the third or fourth day, vesicles on the fourth or fifth, and dry scabs on about the seventh day. The scabs had all fallen off as a rule by about the end of the third week, but in a few cases they remained for a few days longer. Since the appearance of an artificially-induced goat-pox reaction at its height—say on the fifth and sixth days—is not widely known, it may be described by stating that it is almost exactly the same, both as to size of vesicles and nature of their contents, as a vaccinia eruption in sheep. The course of the infection is, however, milder, and fever rarely develops. In the experience of the writers, all goats are susceptible to goat-pox, as all sheep are to vaccinia.

Range of Pathogenicity of Goat-Pox.—The differences of opinion held on this subject are reflected in two summaries recently presented to international congresses. Bridré (loc. cit.) states that goat-pox is pathogenic for the goat only, whether by natural or artificial transmission. He mentions, indeed, that certain workers have claimed to transmit it to sheep but failed to passage it in these animals. He quotes, however, and evidently agrees with other workers who have failed at even primary transmission. Melanidi and Tzortzaki (1936), on the contrary, state that although goat-pox occurs naturally in goats only, it can be artificially transmitted to cattle, horses, dogs, rabbits, and even poultry. Nevertheless, some years earlier [see Blanc, Melanidi and Stylianopoulo (1923-26)], Melanidi collaborated in experiments from which it was concluded that goat-pox was not transmissible to rabbits as well as other animals. Recent original literature on the subject is rare. In Palestine (see most recent Annual Reports of the Chief Veterinary Officer) sheep and goats are said to be almost equally affected in nature, but no experiments upon it seem to have been done. In Turkey (Kolayli, Mavridis and Ilhami, 1933), the mortality due to goat-pox is said to be sometimes high, and in severe outbreaks to amount to from 15 to 40 per cent. Sheep are also said to suffer from the disease, but they do not acquire it naturally from goats because clean sheep crowded in a stable with infected goats remain healthy. Nevertheless the infection can be transmitted to sheep by intradermal injection. It is also said to be non-infective to calf, foal and rabbit.

In the later section on the relation between goat-pox and contagious pustular dermatitis, it is shown that there is much confusion in regard to their differential features, possibly to some extent attributable to differences in the local breeds of animals. However, accepting the material received from Palestine as goat-pox the writers carried out a few tests with concentrated etherised vesicle material. No difficulty was encountered in obtaining typical pox syndromes in sheep, calves, rabbits and monkeys. Except in sheep, the infection was milder and more transient in all these animals than in goats. In calves (eight), papules occurred on about the fifth day and the last scabs had usually fallen by the tenth; in rabbits (eight) and monkeys (three), erythema and papules appeared on about the third day and the skin was clean before the tenth. The reaction of sheep, however, was, in most cases, more acute, although not more persistent, than in goats. Of 18 sheep, four showed marked fever, six mild fever and eight no fever; two which had marked fever also developed subcutaneous oedema, several square inches in area, under the scarified area. In all, the whole eruption cleared up, however, in less than three weeks.

Immunological Relationship.—This seems never to have been worked out in the case of goat-pox and vaccinia. The following experiments were carried out in Khartoum:—

1. Does Vaccinia Protect Against Goat-Pox?

Four sheep and six goats were immunised with vaccinia by scarifying the whole of the abdominal wall with 1/1 etherised vac-

TABLE III
TEST OF IMMUNITY TO GOAT-POX IN SHEEP AND GOATS INFECTED
42 AND 60 DAYS EARLIER WITH VACCINIA

	<i>Animal No.</i>	<i>Febrile Reaction</i>	<i>Local Reaction</i>
Recovered vaccinia sheep (42 days)	58	O	++
	59	+	++++
	60	+	++
	61	O	++++
Control sheep	62	O	++
	63	O	++++
Recovered vaccinia goats (60 days)	1	O	++++
	2	O	++++
	4	O	++
	5	O	++
	6	O	++++
	7	O	++++
Control goats	A	Not recorded	++++
	B		++++
	C		++++

cinia vesicle fluid. All "took" severely. At intervals of 42 and 60 days in the case of sheep and the goats respectively all were tested by equally extensive scarification with 1/1 etherised goat-pox vesicle contents. Immunity to the original virus was not checked, as preliminary tests had shown that a severe "take" with either virus always protects against the corresponding virus. The result is shown in Table III, from which it is obvious that vaccinia does not protect against goat-pox.

2. Does Goat-Pox Protect Against Vaccinia?

Blanc, Melanidi and Stylianopoulo (loc. cit.) had stated that goat-pox differs from all other pox viruses in that it does not protect against itself in goats. In order, therefore, to forestall criticism, one modification was introduced as shown in the last column of Table IV.

Four accumulated sheep and six goats, all of which had been infected with goat-pox by extensive abdominal scarification, were tested for immunity to vaccinia in the usual way. No controls were used, for reasons already explained. The outcome of the experiment would have made them unnecessary in any case. The result is shown in Table IV, which shows that goat-pox did not protect against vaccinia in either sheep or goats.

TABLE IV
TEST OF IMMUNITY TO VACCINIA IN SHEEP AND GOATS INFECTED FROM
37 TO 52 DAYS EARLIER WITH GOAT-POX

	<i>Animal No.</i>	<i>Interval</i>	<i>Febrile Reaction</i>	<i>Local Reaction</i>	<i>Retest with Goat-pox</i>
Recovered goat-pox sheep	13	43 days	++	+++	Not done
	14	43 "	O	+++	
	18	37 "	++	+++	
	20	37 "	++	+++	
Recovered goat-pox goats	8	52 "	++	+++	All showed scurfy "immediate" re- action only. *
	9	52 "	++	+++	
	10	50 "	++	+++	
	11	50 "	++	+++	
	12	42 "	++	+++	
	13	41 "	++	+++	

* This phenomenon will receive further discussion in the section dealing with the relationship of goat-pox to contagious pustular dermatitis.

Summary

The foregoing experiments show that the virus of goat-pox has a wide range of pathogenicity and that there is no immunological relation between it and vaccinia.

RELATION BETWEEN SHEEP-POX AND GOAT-POX

Range of Pathogenicity.—It has already been said that while there is great divergence of opinion in the literature as to the exact

relationship of sheep-pox and goat-pox, our experiments have shown that whereas goat-pox was transmissible to all animals tested, sheep-pox was transmissible to none.

Immunological Relationship.—"Caprinisation" of sheep-pox, which has already been discussed, as originally conceived did not imply any immunological relationship between sheep-pox and goat-pox. All that was presumed was that sheep-pox virus became attenuated by goat-passage while still remaining sheep-pox. Kolayli *et al.* (loc. cit.) claim that a similar attenuation of goat-pox for goats can be effected by passage through sheep. In addition, however, they state that sheep recovered from goat-pox are immune to sheep-pox. Their conclusions are worthy of full quotation in original:—

La conclusion de nos travaux sur la variole est la suivante :

1° Considérant que le virus de la variole des chèvres ne se transmet pas par contagion naturelle des chèvres aux moutons, et que ce virus, inoculé aux moutons, après production d'une pustule bénigne, les immunise contre leur propre clavelée, l'inoculation du virus variolique des chèvres contre la clavelée du mouton est un système qui, tout en immunisant les moutons, dissipera en même temps le danger de propagation du mal consécutif à la pustule produite par le virus des moutons claveleux.

2° Si l'on utilise le claveau dans la vaccination des chèvres exposées à la contagion en temps de variole épidémique, on pourra éviter la production des accidents de généralisation quelquefois observés résultant de l'application du vaccin obtenu des chèvres varioleuses.

The first of these conclusions definitely implies immunological relationship, and as such calls for investigation: the second is an extrapolation therefrom. Experiments carried out to check the claim were as follows:—

1. *Does Sheep-Pox Protect Against Goat-Pox?*

A few sheep recovered from sheep-pox had already been found fully susceptible to goat-pox. It remained to confirm these preliminary observations by testing the immunity of several more, both natural and experimental cases. The goats employed could only be described as "recovered" from sheep-pox in the sense that they were goats to which transmission had been unsuccessfully attempted by multiple intradermal injections of 1/1 virus suspension. No controls were used, as the preliminary observations had shown that they would be unnecessary. Finally, 13 sheep and nine goats were tested (Table V).

The table shows conclusively that sheep recovered from sheep-pox are not protected against goat-pox. It also illustrates, incidentally, the observation already recorded that sheep react more severely than goats to goat-pox infection. Little significance attaches to the

TABLE V
TEST OF IMMUNITY TO GOAT-POX IN SHEEP RECOVERED FROM NATURAL
AND ARTIFICIAL SHEEP-POX, AND IN GOATS INJECTED WITH CONCENTRATED SHEEP-POX
VIRUS

	<i>Animal No.</i>	<i>Interval</i>	<i>Febrile Reaction</i>	<i>Local Reaction</i>
Sheep recovered from natural sheep-pox	13	32 days	+	++
	14	32 "	O	+++
	17	49 "	++	+++
	27	30 "	+	+++
	28	30 "	+	+++
	29	30 "	++	+++
Sheep recovered from artificial sheep-pox	19	49 "	+	+++
	21	49 "	+	+++
	24	57 "	++	+++
	25	57 "	++	+++
	26	57 "	O	+++
Goats	1	94 "	O	+++
	2	94 "	O	+++
	4	85 "	O	+++
	5	85 "	O	+++
	6	77 "	O	+++
	7	77 "	O	+++
	14	17 "	O	+++
	15	17 "	O	+++
	16	7 "	O	+++

goats, and they were only included in the test because they happened to be on hand.

2. Does Goat-Pox Protect Against Sheep-Pox?

This could only be tested in sheep, because local goats cannot be infected with sheep-pox. A special experiment, complete with controls, was carried out, immunity being tested 38 days after infection. The result is shown in Table VI, which shows quite plainly that goat-pox does not protect sheep against sheep-pox.

TABLE VI
IMMUNITY TO SHEEP-POX IN SHEEP INFECTED 38 DAYS EARLIER WITH GOAT-POX

	<i>Sheep No.</i>	<i>Febrile Reaction</i>	<i>Local Reaction</i>
Sheep recovered from goat-pox	22	++	+++
	23	++	+++
	30	++	++
	31	+	O
	32	+++	+++
Control sheep	52	+	+++
	53	++	++
	54	++	O
	55	+	+++
	56	O	+
	57	O	O

Summary

The pathogenic range of sheep-pox and goat-pox has already been discussed. Work recorded in this section shows that there is no immunological relationship between the two viruses.

RELATION BETWEEN GOAT-POX AND CONTAGIOUS PUSTULAR DERMATITIS

The term contagious pustular dermatitis is used because it was selected by Glover (loc. cit., 1931) as the most suitable of the numerous designations under which this affection is known (contagious pustular stomatitis, contagious ecthyma, vesicular verrucose eruption of the lips, etc.). There has been a great deal of confusion between this disease and goat-pox, especially in countries where both exist, the clinical symptoms in natural outbreaks being not always easy to differentiate. In outbreaks of goat-pox where generalisation occurs the two diseases are fairly well distinguished, but in mild outbreaks where lesions are limited to the region of the muzzle, the difficulty is considerable. The eruptions of contagious pustular dermatitis have not always been recorded as limited to the muzzle and mouth. The word "dermatitis" in place of the original "stomatitis" is therefore more preferred when referring to this disease. The previously alleged specificity of goat-pox for the goat must also have aggravated the confusion.

The two extremes of opinion are best illustrated by two publications on the subject. Blanc, Melanidi and Stylianopoulo (loc. cit.) doubt whether goat-pox really is a specific disease; they recognise contagious pustular dermatitis, but are not sure that so-called goat-pox is not merely "*un syndrome dû à des causes différentes.*" On the other hand Slagsvold (1938) says the two conditions are identical, Slagsvold's "vesicular verrucose eruptions" being presumed to be what would now be termed contagious pustular dermatitis.

The relationship of these two diseases needed clearing up, for otherwise doubt would remain as to whether the virus used in our preceding experiments was really that of goat-pox. As the clinical syndrome in natural infection is a very uncertain criterion, it seemed that the experimental technique that had given such clear-cut results with the other pox viruses, might serve to differentiate the two viruses. A sample of contagious pustular dermatitis material (dry scab) of British origin was therefore obtained from Glover, whose co-operation is gratefully acknowledged.

Experimental Lesions.—The first attempt to implant Glover's virus on to local goats impressed us that we were dealing with something different from the goat-pox received from Binns in Palestine. Two goats scarified over a wide area of belly and thighs with the original material after the usual ether treatment developed a very light transient dry "take" along the lines of scarification. The papular and vesicular stages, which seemed to develop on the third and fourth days, respectively, were almost impossible to detect. All

that could be seen on the fifth day was a fine, continuous, dry scab along each line. There was uncertainty in fact as to whether the virus had really taken. The scabby material was, however, scraped off on the fifth day, etherised, and scarified on to another goat which developed a rather more marked local reaction. There was no erythema, but a distinct linear thickening, corresponding to the papular stage, was visible along the scratches on the fourth day, and a few isolated white papules were present between the lines. The papular stage passed directly into the scab stage on the next day without any vesicles having formed. Material taken from part of this eruption on the fourth day was etherised and scarified on to two further goats. These developed a slightly more marked eruption, as there was distinct erythema on the third day before the papular stage appeared on the fourth; otherwise the syndrome was as that of the second passage. A fourth passage was made on three goats, and, as more material was available, a series of dilutions from 1/1 to 1/1,000 was tested. One of the three goats developed a few frank vesicles, about 2 mm. in diameter, on the 1/1,000 area on the fourth day, but otherwise the affection was as at the previous passage. The goats were left unscraped for continued observation, and the last scabs fell on the 16th, 17th and 18th days, respectively. Many goats subsequently scarified behaved in an exactly similar manner, no further exaltation being noticed. No fever occurred in any goat.

The material received from Glover had been recovered from sheep, and so it was at first thought that the three goat-passages necessary to obtain the maximum reaction in goats might be evidence that the virus needed reaccommodating to these animals. Such, however, proved not to be the explanation, as was shown by a later experience. On this occasion pulp scraped from goats had been desiccated *in vacuo* over phosphorus pentoxide in a refrigerator, sealed in exhausted ampoules, and then stored for two months in a refrigerator. When etherised and scarified on to fresh goats, exactly the same phenomenon recurred; three passages were necessary before maximum local response could be reproduced. This was striking evidence of the danger of using slightly stale material in experiments on contagious pustular dermatitis.

The foregoing behaviour was in marked contrast to that of the goat-pox material received from Palestine. This constantly produced marked erythema from the first; and well-filled vesicles, noticeably larger than those of contagious pustular dermatitis, always intervened between the papular and scab stages. No difference, moreover, was ever observed between reactions to freshly gathered multiple-passaged material and to that which had been stored for some months.

These observations, and many subsequent ones that corroborate them, demonstrate that, whatever may be said of the natural lesions of these two diseases in goats, the experimental lesions differentiate them quite unequivocally. Systemic symptoms, however, are of no differential value, since although no goat has yet been observed

to develop fever when artificially infected with contagious pustular dermatitis, very few have done so when similarly infected with goat-pox.

Range of Pathogenicity.—The range of goat-pox, already discussed, can be summarised by saying that not only was it found to be transmissible to all animals tested, but it affected sheep more acutely than goats.

Although there is a fair amount of contradictory evidence, contagious pustular dermatitis is also generally recognised to have a wide range. However, Glover (loc. cit., 1931), surprisingly, has elicited from the literature that “apart from some isolated experiments by Lanfranchi and by Altara, there is general agreement that the rabbit, an animal which is admirably suited to the detection of the virus of vaccinia, is entirely refractory to the virus of dermatitis.” Glover seems to conclude that non-transmissibility to the rabbit is about the only reliable fact that stands out from a mass of otherwise equivocal evidence.

In pathogenicity tests carried out in Khartoum with freshly passaged, concentrated, purified virus, no difficulty was experienced in effecting transmission to calves (six), rabbits (six), and monkeys (two), the only animals tested. It was, however, the character of the lesions in these animals, and not the mere fact of their susceptibility, that proved of special interest. In calves the lesions were not significantly different from those of goat-pox, although they were slightly drier and lighter. The rabbits and monkeys, on the other hand, behaved quite distinctively.

In the first two rabbits tested, the eruption resembled goat-pox in passing directly from papules to scabs without a frank vesicular stage, but the whole eruption seemed to be coarser and more haemorrhagic than that of goat-pox. To ensure that the observed difference did not fall within the range of normal variation, four more rabbits were scarified with both viruses, one on each side of the shaved abdomen. Insertion was effected by gently scarifying the whole of the shaved area with the freshly cut edge of a small piece of fine (40 mesh) brass wire gauze held in artery forceps. The papular stage of both eruptions appeared on the third day, but on this and the succeeding day the two lesions unequivocally differed in appearance. In all cases the goat-pox eruption consisted of a pale cream-coloured, light, filmy crust, evenly distributed over the scarified area. There was no general erythema, but each rabbit had about half a dozen small haemorrhagic spots, barely 1 mm. in diameter, scattered about in the lesion. The contagious pustular dermatitis eruption was coarser, somewhat darker yellow, and duller looking. Appreciable erythema showed through and around the edges of the crust, and each rabbit had a slightly greater number of definitely larger haemorrhagic spots, better described as blotches, averaging 3 mm. in diameter, scattered about in the lesion. From the fifth day onwards no difference could be noticed: the goat-pox seemed

to "catch up with" the contagious pustular dermatitis, and by the tenth day all scabs had fallen in both cases.

In monkeys the lesions of contagious pustular dermatitis differed even more widely than in rabbits from the rapidly developing, dry, transient eruption of goat-pox. Two monkeys were infected, one with material from the second, and the other with virus from the third, of the goat passages from the stored goat material already mentioned. Both were scarified with 1/1 suspension on six well spaced, parallel lines running the whole length of the abdomen. The second of these monkeys, having been infected with the more exalted goat virus, developed the more severe reaction, and may be described first. The differential characteristics of the local eruption were its slow rate of development, its severity, and its persistence. Small papules could be made out as early as the third day, but by the fifth day they had grown only to the size of a pin-head; where confluent they formed little raised ridges 1 to 2 mm. wide. On the sixth day intense erythema appeared, having perhaps been earlier masked by the blue skin of the monkey. Thence, until the 10th day, the deeply congested confluent papules slowly grew to form prominent purple ridges 4 to 5 mm. in diameter. Here and there some umbilication could be seen, but there were no frank vesicles. From the 11th day the papular mass progressively softened, until by the 15th day it was completely pustular and enclosed by a thin hard shell. From this point the pustules became slowly filled with granulation tissue, until by about the 20th day the ridges could be described as solid granulomatous bars. During the next three weeks the granulation tissue dried, crumbled, and fell off piecemeal, until at the end of the seventh week nothing remained but clean, white, raised scars. The other monkey, which had been similarly scarified with material from the second goat-passage, developed only four widely spaced lesions, but these went through exactly the same stages. During the fourth and fifth weeks they had the appearance of warts, about a centimetre in diameter, after which they crumbled away and disappeared during the sixth week.

Thus, while the range of pathogenicity in unnatural hosts, so far as this was tested, was the same in both viruses, the character of the lesions in rabbits, and even more so in monkeys, was markedly differential.

Immunological Relationship.—The literature is particularly uninformative on this point. Blanc, Melanidi and Stylianopoulou (loc. cit.) state that contagious pustular dermatitis, while immunising against itself, does not immunise against goat-pox, and that goat-pox immunises neither against contagious pustular dermatitis *nor against itself*. The last observation, it will be recalled, leads them to question whether goat-pox is a specific disease or a non-specific syndrome. The possibility might be added that they were not dealing with goat-pox at all. Roe (1932) reports from Cyprus that "dried scabs from a kid which was naturally affected with this disease (C.P.D.)

produced a fairly typical reaction when inoculated into the lips of a goat which was immune to goat-pox." Glover (1933) records cross-immunity tests carried out with British contagious pustular dermatitis material and scabs sent from Cyprus which were thought by Roe probably to be of goat-pox. Using only one animal for each test, he reports having effected appreciable, but not complete, cross-immunisation in both directions, and concludes that the Cyprus material was probably contagious pustular dermatitis.

The above evidence introduces two new features that have not arisen where the other poxes are concerned, *viz.*, the suggestion that a pox may not immunise against itself, and the significance of partial cross-immunisation. The experiments now to be recorded had therefore to take these features into account, so that in all immunity tests each animal had to have its immunity to the first immunising virus checked, and the test virus had to be inserted in a range of dilutions. At the time of testing immunity, therefore, both viruses were inserted into each animal in dilutions of 1/1, 1/10 and 1/100. The 1/100 dilution was selected as the highest to test because it had already been observed that even clean goats developed lesions of varying intensity in dilutions of 1/1,000 and upwards, whereas at 1/100 and downwards all developed confluent or semi-confluent "takes." Variation in intensity of reaction to dilutions of 1/1,000 and upwards has, in fact, no immunological significance, and the use of high dilutions would have been confusing rather than helpful.

Although cross-immunity tests in both directions were carried out simultaneously, for the sake of having one set of controls to both viruses, the two are best recorded separately:—

1. Does Goat-Pox Protect Against Contagious Pustular Dermatitis?

Four goats, recovered from heavy artificial infection with goat-pox, were tested with 1/1 to 1/100 contagious pustular dermatitis suspensions scarified on to three oblong areas, about 8 × 5 cm., on one side of the abdomen. At the same time similar areas on the other side were scarified with goat-pox in order to check immunity to this

TABLE VII
IMMUNITY TO CONTAGIOUS PUSTULAR DERMATITIS IN GOATS RECOVERED FROM
GOAT-POX

Goat No.	Interval	Febrile Reaction	Local Reaction (all Dilutions)	Goat-pox Check-test (all Dilutions)
22	103 days	O	O	O
29	30 "	O	O	O
30	30 "	O	O	O
31	30 "	O	O	O
36	Controls	O	+++	+++
37		O	+++	+++
38		O	+++	+++

virus. The result is set out in Table VII, which shows that goat-pox immunised completely against both itself and contagious pustular dermatitis.

2. Does Contagious Pustular Dermatitis Immunise Against Goat-Pox ?

Eight goats, recovered from artificial contagious pustular dermatitis infection, given at intervals between 28 and 50 days earlier, were similarly tested with a range of goat-pox dilutions, and at the same time were checked as to their immunity to the original contagious pustular dermatitis. The results are shown in Table VIII.

TABLE VIII
IMMUNITY TO GOAT-POX IN GOATS RECOVERED FROM
CONTAGIOUS PUSTULAR DERMATITIS.

Goat No.	Interval	Febrile Reaction	Local Reaction			C.P.D. Check Test (all Dilutions)
			1/1	1/10	1/100	
24	50 days	O	+++	+++	++	All showed scurfy "immediate" re- action only.
25	50 "	O	+++	+++	+++	
26	43 "	+	+++	+++	++	
27	36 "	O	+++	+++	+++	
28	36 "	O	+++	+++	++	
32	28 "	O	+++	+++	++	
33	28 "	O	+++	+++	+++	
34	28 "	++	+++	+++	++	
36	Controls	O	+++	+++	+++	+++
37		O	+++	+++	+++	+++
38		++*	+++	+++	+++	+++

* This goat is shown as "O" in the same column of Table VII as it acted as control to both experiments. The fever is presumed due to goat-pox.

This table shows that contagious pustular dermatitis, while protecting against itself, does not protect against goat-pox.

The "immediate" reaction noticed in all goats on the area re-scarified with contagious pustular dermatitis recalls a similar phenomenon recorded in Table IV, but on that occasion it occurred in goats scarified a second time with goat-pox. This type of reaction, which in human beings revaccinated with vaccinia is recognised as a sign of complete immunity, has received little or no attention in animal pox infections. Gins and Kunert (loc. cit.) claim to have noticed it in varying degree, and invoke it as proof of strong immunity, in sheep immunised by nasal insufflation of "ovinised" vaccinia and subsequently scarified with a non-ovinised strain. They also claim to have observed it, in even more widely varying degree, in the same sheep subsequently tested for immunity to sheep-pox by nasal instillation of virus. These observations, however, in common with many others recorded in their paper, are not at all convincing. In particular, attention may be drawn to the acceptance

of pustule formation as a character of some of the above-mentioned immediate reactions. In a revaccinated human being, pustule formation would at best indicate a low level of immunity (vaccinoid), and might even indicate complete absence of immunity. The immediate reactions of our own goats, both to goat-pox and to contagious pustular dermatitis, showed exactly the same series of changes as those regularly observed in human beings immune to vaccinia, namely, very slight erythema, with or without rudimentary papule formation, occurring within 48 hours, passing on the third day, without pustule formation, into a mere piling up of desquamated epithelium that could easily be rubbed off with the finger tips. It is, further, interesting to note that immediate reactions to goat-pox occurred in all goats retested in Table IV but none occurred in any goat similarly retested in Table VIII. No explanation is offered, but it is presumed that some slight difference in the constitution of the inoculum, which was merely etherised vesicle material and not washed E.B.s was responsible. However, having observed the phenomenon, which is not generally known to occur, one cannot help wondering whether Blanc *et al.* (loc. cit.), who used unetherised scab material in their experiments, did not interpret a combination of immediate reaction and impetigo as a pox lesion. If so, it might explain how the goat from which they drew their conclusions developed what were taken as pox lesions, not only after a second, but also after a third, scarification with unetherised scab emulsion.

The results of the cross-immunity tests summarised in Tables VII and VIII show that goat-pox immunised completely against contagious pustular dermatitis, while the latter did not immunise even partially against goat-pox. If neither virus had immunised against the other, the experiments would at least have confirmed what the transmission tests had shown, namely, that the two viruses are different. Now, however, the relationship as well as the difference is clear. For the first time, outside the variola-alastrim-vaccinia group, two mammalian pox viruses have been shown to possess a close antigenic relationship. Goat-pox, while including all factors present in contagious pustular dermatitis, obviously incorporates at least one factor that is missing from the latter.

Finally, his conclusion will explain Roe's observation that a goat recovered from what he took to be goat-pox was susceptible to contagious pustular dermatitis. What evidently occurred was the reverse, as Glover's identification of his suspected goat-pox material as contagious pustular dermatitis confirms. Roe correctly observed that he had to deal with two different diseases, but had no means at his disposal of definitely identifying them.

Summary

The important points of contrast in the properties of the virus of goat-pox and contagious pustular dermatitis are:—

Judging from the literature (since the writers have no first-hand experience), the clinical manifestations of the naturally occurring diseases are different, goat-pox being the more severe. However, mild outbreaks of goat-pox and severe ones of contagious pustular dermatitis are apt to overlap the normal boundary line and make differentiation difficult or impossible.

Experimental infection in the commoner of the two natural hosts, namely the goat, serves to differentiate the two conditions.

The pathogenic range in unnatural hosts appears to be the same in both cases, observations being limited to species of animals commonly used in laboratory work on poxes. Both viruses infected all animals tested.

As in the case of the natural host (goat), so in some unnatural hosts (rabbit and monkey) the character of the experimental lesions served to differentiate between the two infections.

Cross-immunity tests showed that while goat-pox protected against contagious pustular dermatitis in goats, no protection was afforded in the reverse direction. The two viruses are therefore closely related, goat-pox possessing at least one antigenic factor that contagious pustular dermatitis lacks.

DISCUSSION

The results obtained in the foregoing experiments confirm the writers in their original view that the divergence of opinion that has hitherto existed regarding the range of pathogenicity and the immunological relationships of the pox diseases of sheep and goats (and probably also those of other animals) has in no small measure been due to unsuitable experimental technique.

Animal poxes are usually distinguished from each other not so much by their clinical syndromes in naturally occurring outbreaks, in which they are apt to be variable and not entirely differential, as by their range of pathogenicity in experimental animals and their cross-immunity relationships. It would, however, seem that studies of these properties have been attended by equivocal results, largely on account of scab material having been used as a source of virus. Scabs, as is becoming generally recognised by workers on the variola-alastrim-vaccinia group of infections, are notoriously apt to be poor in virus, even when freshly gathered. In addition they always contain numerous bacteria; these, in the pox diseases in which experimental skin eruptions are light or transient, are liable to produce an impetigo that will prejudice the accurate assessment of more or less pox-like lesions. The use, in the experiments recorded in this paper, of infective material gathered in the papular or vesicular stage, and treated with ether to eliminate pyogenic bacteria, has afforded results that have been not only clear-cut, but also entirely consistent in very many animals included in several experiments.

Another item of technique to which it is relevant to draw atten-

tion in this discussion is the necessity for adopting the intradermal route in infection experiments with sheep-pox, since scarification has proved unreliable even when freshly prepared E.B. suspension has been used as the inoculum. The remaining items, namely, site of infection, area of skin scarified, interval between immunisation and immunity tests, and the use of isolated premises for each virus, have been sufficiently discussed in the body of the paper and need no further attention here.

Since the immunological interrelationship of any two viruses must necessarily be the ultimate criterion of identity, and since such entirely satisfactory results have now been obtained in this respect, closer discussion of the range of pathogenicity is unnecessary. Two points, however, deserve brief mention, namely, the breed of animal used, and the character of the lesions produced. No observations made in the course of the present work have a direct bearing on the former, but it is relevant to draw attention to the increasing importance that is now being attached to the point in experimental work on viruses generally. In the clinical sphere, moreover, the wide variation in the behaviour of different types of sheep towards sheep-pox has long been recognised. The difficulty of correlating the results of workers in different parts of the world would be almost insuperable, and it is fortunate that range of pathogenicity is a feature of only secondary importance being automatically superseded by immunological evidence when such becomes available.

The character of the lesions produced in susceptible animals may, however, be of greater importance, and may yet serve to produce useful evidence of the identity of certain poxes in which cross-immunity tests are difficult to carry out for one reason or another. It is remarkable, in consideration of the importance that has been attached in the past to the transmissibility of a pox to an experimental host, that so little notice has been taken of the character of the infection when once transmitted. Possibly, owing to secondary complications produced by the bacterial infections already adduced as the cause of so much dispute, sufficient uniformity has not been observed to attract attention to the point. Two examples of the importance of the character of experimental lesions stand out very clearly in the present work where differentiation of goat-pox from contagious pustular dermatitis is concerned: the local lesions in rabbits, and even more so in monkeys, were markedly different in the two infections. It was also observed that sheep, as a rule, develop distinct fever when infected with goat-pox, but it cannot definitely be said that they fail to do so when infected with contagious pustular dermatitis because they were not tested. However, the general literature on the subject seems to show that sheep artificially infected with the latter disease do not develop fever.

Particular attention is drawn to goat-pox and contagious pustular dermatitis because there has probably been more doubt about their

relationship than about that of any other pair of infections. It might still be argued, against the writers' conclusions, that the condition here accepted as goat-pox is so closely identical in its range of pathogenicity, and so closely related immunologically, that it is no more than a "type" of contagious pustular dermatitis. The reply to this argument is: firstly, that the two naturally occurring infections are, in the main, clinically distinct, although there may occasionally be some overlapping of syndromes; secondly, that although the mere range of experimental pathogenicity is, so far as it has been studied, identical, nevertheless in the rabbit, and even more so in the monkey, the character of the skin eruptions is absolutely distinctive; and thirdly, that although the two infections are immunologically so closely related they are also antigenically distinct. It is therefore proposed that until some pox disease of the goat, conforming in its naturally occurring clinical manifestations to the orthodox descriptions of goat-pox, is found to be immunologically less closely related to contagious pustular dermatitis, the virus studied in the foregoing experiments should be recognised as that of goat-pox.

This paper is primarily concerned with the status of the viruses studied, and not with the changes that may be effected in them by the well-nigh boundless number of manipulations to which they can be submitted in unnatural hosts. Nevertheless, observations have been made on the so-called "caprinisation" of sheep-pox and "ovinisation" of vaccinia. It is to be noted, by the way, that these two processes are not homologous, in spite of verbal similarity, the former being postulated as a mere attenuation, and the latter as an immunological transmutation. That it was found impossible to effect "caprinisation" of sheep-pox may have been due merely to the relative insusceptibility of the local breed of goat, and even if it had been effected there would have been no question of refuting a claim that the virus had been immunologically altered: such a claim has never been made, but the writers, in spite of the uniform susceptibility of local sheep, have been unable to confirm it.

The remaining items call for no special discussion, being merely records of observations open to only a single interpretation.

SUMMARY

The character of sheep-pox in the Sudan is discussed. The disease appears to be enzoötic in the country, but has hitherto escaped notice because, although sometimes affecting individual sheep severely, it is usually mild, and spreads so slowly that it never becomes epizöotic.

The literature relating to the pox diseases of sheep and goats is reviewed, and it is concluded that the divergence of opinion that exists about their interrelationship is largely due to unsuitable technique in carrying out susceptibility and cross-immunity tests.

Certain improvements in technique are recommended, and their suitability confirmed by the clear-cut results obtained in the extensive series of infection experiments recorded.

The results of experiments carried out with the improved technique lead unequivocally to the following conclusions regarding the relationships of the viruses studied.

1. *Sheep-Pox and Vaccinia*.—Vaccinia has a wide range of pathogenicity. Sheep-pox, conversely, is not *ordinarily* transmissible to any animal other than sheep. There is no immunological relationship between the two, and even enormous doses of the one will not protect against the other. The hypothesis that vaccinia can be "ovinised," *i.e.*, immunologically transmuted by sheep-passage into sheep-pox, is not confirmed. The hypothesis that sheep-pox can be "caprinised," *i.e.*, attenuated by goat-passage, could not be studied because infection could not be established in goats.

2. *Goat-Pox and Vaccinia*.—There is no difference in the range of pathogenicity so far as sheep, calves, rabbits and monkeys are concerned. The skin eruption of goat-pox is milder than that of vaccinia in all these animals except sheep. In sheep it is indistinguishable from that of vaccinia. Sheep also develop considerable fever, as in vaccinia. There is no immunological relationship between the two.

3. *Sheep-Pox and Goat-Pox*.—The ranges of pathogenicity differ, goat-pox being transmissible to numerous animals (see (2) above) and sheep-pox to none. There is no immunological relationship between the two.

4. *Goat-Pox and Contagious Pustular Dermatitis*.—The ranges of pathogenicity are identical (subject to the limitations of (2) above). The characters of the lesions are by no means identical. The contagious pustular dermatitis virus becomes of very low pathogenicity for the (Nubian) goat after even brief storage under ideal conditions, and needs a few goat-passages to restore it to its normal level, whereas the goat-pox virus seems to maintain a more constant level of virulence. In the rabbit, and even more so in the monkey, the two skin eruptions are easily distinguishable. The two viruses are immunologically related: while contagious pustular dermatitis fails to immunise, even partially, against goat-pox, the latter immunises completely against contagious pustular dermatitis. The goat-pox virus thus possesses at least one antigenic factor which the virus of contagious pustular dermatitis lacks.

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EXPERIMENTS ON THE TRANSMISSION TO THE RABBIT OF THE VIRUS OF CONTAGIOUS PUSTULAR DERMATITIS OF THE SHEEP

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INTRODUCTION

CONTAGIOUS pustular dermatitis is a virus disease of sheep that has been reported from nearly all parts of the world. In this country, Glover (1928) has investigated several outbreaks and has given a full account of both the natural and the experimental disease. The condition usually occurs in outbreaks among lambs during the spring and summer months. It does not lead to a high mortality but it causes a considerable loss in condition which is particularly serious in lambs that are being fattened for the market. The lesions, which commence as discrete vesicles or pustules, are usually confined to the nostrils and lips, and particularly to the commissures. The primary eruption is succeeded by spreading erosions which bleed readily and are covered by exudate which later dries to form thick yellowish-black crusts. In most cases the crusts are shed and the ulcerations heal leaving no trace of the lesion. Sometimes, however, the epithelium proliferates and forms dense wart-like outgrowths which persist for several weeks but are finally resorbed. As will appear, this wart-like form was the type of infection that was present in the lamb from which was derived the strain of the virus of contagious pustular dermatitis to be described here.

EXPERIMENTAL

The Natural Infection in Lamb 1

On March 21st, 1936, R. L. Greenhalgh, M.R.C.V.S., of Lancaster, sent a specimen to the laboratories of the Imperial Cancer Research Fund for histological examination. The specimen was a warty growth that had been removed from the lower lip of a three-weeks-old cross-bred lamb and was one of several lesions that had appeared on the lamb's lips and nostrils during the previous few days. The microscopical appearance of the growth suggested that it was caused by a virus and the infective nature of the condition was confirmed by Mr. Greenhalgh, who found that 19 out of 52 lambs were affected and that, in the district concerned, there had been occasional outbreaks of a similar nature.

In view of the possible relationship that this condition might have to papillomatous growths, and hence to the virus aetiology of epithelial tumours, Dr. W. E. Gye arranged to have the lamb sent up for further investigation. When the original lamb (Lamb 1)

arrived on March 28th it was found to have low warty lesions over the lips and the lower border of the left nostril. On the upper lip the lesions were confluent, dark, and mostly covered with crusts, and all had a great tendency to bleed. On the lower lip the lesions were flat and discrete and had a rough warty surface. During the next ten days the lesions were evidently regressing and on April 8th they were all removed and stored in 50 per cent. glycerine. A further examination on May 4th showed that the lesions had healed completely.

TRANSMISSION EXPERIMENTS

Transmission to Lambs and Kids

Lambs 2 and 3, 23.4.36. Glycerinated material from Lamb 1 was washed in three changes of saline, ground with sand, and made up to a 5 per cent. extract with saline. The extract was centrifuged at 4,000 r.p.m. for 20 minutes and a few drops of the supernatant fluid were rubbed into the left upper lip and a shaven area of the nape of Lambs 2 and 3 after scarification with a sharp needle.

When the lambs were examined a week later the inoculated areas on the nape had thick crusts of exudate mixed with a matted growth of wool, while the lips showed exudative lesions with discrete papules at the periphery. At the end of a fortnight the nape lesions had become thick fissured masses of exudate intermingled with scales and matted wool. The lip lesions had now become drier, taking on a warty appearance, and in Lamb 3 there were also numerous vesicles at the periphery. Most of the lesions were removed from Lamb 2 on the 18th day and from Lamb 3 on the 22nd day after inoculation. When the lambs were examined 30 days after inoculation the lesions had completely healed.

Lambs 4 and 5, 22.5.36. Lambs 4 and 5 were inoculated by scarification on the skin of the back with 10 per cent. extracts and Berkefeld filtrates of glycerinated material from Lambs 1 and 3 (stored 44 and 7 days, respectively). At the end of a week all the inoculated areas showed exudative lesions with matting of new-grown wool. No differences were detected between areas inoculated with material from Lambs 1 and 3 or between areas inoculated with crude extracts and Berkefeld filtrates. The lesions were observed for 19 days after inoculation, during which period their progress was similar to that of the lesions already described in Lambs 1 and 2.

Kids 1 and 2, 22.5.36. Two kids were inoculated by scarification on shaved areas of the flanks with the same extracts as were used for Lambs 4 and 5. A week later there were flat, brownish, scaly nodules, varying in diameter from 2 mm. to 2.5 cm. on all the inoculated areas. During the succeeding week these nodules increased in size and became confluent, forming thick nodular cakes with a rough scaly surface. The lesions were still in the progressive phase when they were examined 19 days after inoculation.

Transmission to Rabbits

Rabbits 1, 2 and 3, 3.6.36. A 10 per cent. spun extract of glycerinated material from Lamb 3 (stored for 19 days) was inoculated by scarification on shaved areas of the backs of three rabbits. Twenty days later discrete, erythematous, scaly patches were noted on the inoculated areas of all three rabbits. Rabbit 1 was killed on the following day and the lesions were stored in 50 per cent. glycerine.

The work was transferred at this stage to the Bland-Sutton Institute of Pathology and no further observations were made on the two remaining rabbits of this experiment.

Rabbits 4 and 5, 30.6.36. Two laboratory stock rabbits of the Bland-Sutton Institute of Pathology were inoculated by scarification on the skin of the flanks with spun extracts and Berkefeld filtrates of glycerinated material from Lamb 3 and Rabbit 1 (stored for 46 and 6 days respectively). After an interval of 20 days scaly and erythematous patches were noted in all the areas that had been inoculated with extract or filtrate of the rabbit material. Similar lesions were also produced in Rabbit 5 by both the extract and filtrate of the lamb material, but they did not appear till after 25 days and they were much less severe than those arising from rabbit material in the opposite flank.

Attempted Transmission to Guinea-pigs, Rats and Mice

Two guinea-pigs, 10 rats and 20 mice were inoculated by scarification on the epilated skin with 19-day-old glycerinated material from Lamb 3, along with Rabbits 1, 2 and 3. None of the animals showed any reaction during an observation period of 21 days.

THE EXPERIMENTAL INFECTION IN RABBITS

Further successful passages of the virus were obtained from the lesions in Rabbits 4 and 5 at intervals after inoculation of 30 and 45 days respectively. The latter passage provided the material for the subsequent study of this virus strain which is now in its 19th rabbit passage after alternate periods of passage through rabbits and storage either in 50 per cent. glycerine or in the dried state.

The details of the properties of the virus and the results of its passage through rabbits will be described in a later communication, but a general description of the infection based on the findings in over 90 rabbits will be given here.

Incubation Period

During the first five passages through rabbits the incubation period was reduced from 20 to 7 days. In later passages the incubation period varied and appeared to depend largely on the source of the infecting material. After the inoculation of glycerinated material lesions usually appeared about the 14th day but

might appear at any time from the 7th to the 19th day. With extracts of fresh material the incubation period was frequently as short as 6 days but might be as long as 19 days. This variation was apparently associated with the age of the lesions used for passage, for when rabbits were inoculated with extracts of lesions of less than 14 days' duration the average incubation period was 9 days, whereas with extracts of older lesions the average incubation period was 13 days.

Course of the Infection

The first evidence of infection is the presence of slightly scaly erythematous patches on the inoculated skin. During the next few days the erythema deepens and usually becomes confluent while the epidermis becomes covered with a layer of fine scales. At this stage a slight thickening of the skin can be detected and during the next 10 to 14 days this thickening increases progressively so that the lesions take on a nodular appearance. The scaliness of the surface of the skin also increases, and this, along with the renewed growth of fur, produces a characteristic scurfy appearance. The lesions by this time have become heavily infected by pyogenic bacteria so that they eventually form thick nodules which bleed readily and usually contain pockets of thick pus. Superficially, the nodules have a craggy appearance and are covered by an infected mass of matted fur, scales, and exudate (Fig. 1). Five to eight weeks after inoculation the nodules begin to drop off, leaving a scaly and slightly thickened epithelium, sometimes with small ulcerations which heal rapidly. Complete regression of the lesions may take 7 to 14 days so that the duration of the disease from the time that it becomes apparent may be 5 to 9 weeks with an average of 7 weeks.

HISTOLOGY

The Naturally Acquired Infection

A section of the naturally acquired lesion from Lamb 1 is shown in Fig. 2. It has the general appearance of a squamous cell papilloma, but it consists of diverging columns of epithelial tissue surrounded by connective tissue instead of having the usual papillomatous form of epithelial outgrowths with a connective tissue core. The epithelial columns consist of squamous epithelium with peripheral basal cells differentiating towards the centre and appear to be hyperplastic hair follicles, some of them containing hair shafts. The epithelial tissue is in active proliferation, many mitoses being found under a higher magnification, and in parts seems to be invading the surrounding tissues. The connective tissue is very vascular and is heavily infiltrated by mononuclear cells and to some extent by polymorphonuclear leucocytes particularly near the surface where the lesion is breaking down.

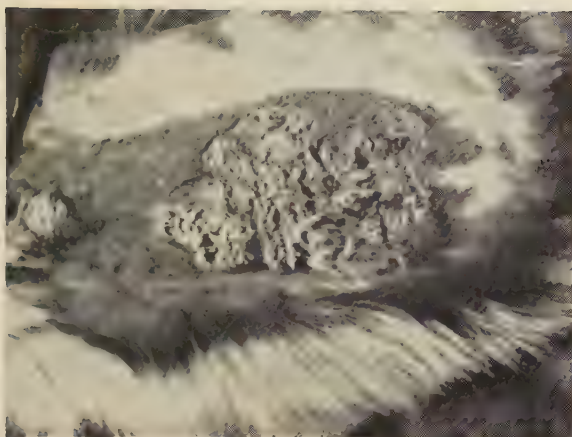


FIG. 1.—Lesion on the flank of a rabbit of the 16th rabbit passage, 18 days after scaliness and erythema were first noted and 26 days after inoculation with an extract of rabbit lesions that had been stored in 50 per cent. glycerine for 322 days.

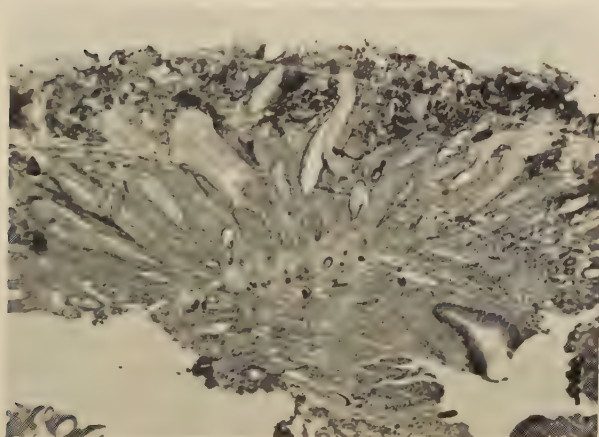


FIG. 2.—Section of the original naturally acquired lesion from the lower lip of Lamb 1. The lesion consists of radiating hyperplastic hair follicles embedded in a vascular and densely infiltrated connective tissue. Frozen section, haemalum and eosin, $\times 6.5$.

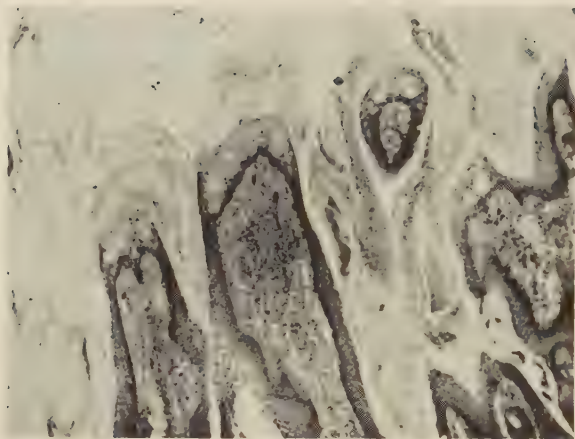


FIG. 3.—Lamb 4, section of skin, 19 days after inoculation with a Berkefeld filtrate of seven-day-old glycerinated lesions from Lamb 3. The hyperplasia of the epidermis and hair sheaths has produced an elongation of the papillae giving the general appearance of a papilloma. At the upper ends of the papillae there are multiloculated intra-epidermal vesicles. Frozen section, haemalum and eosin, $\times 30$.

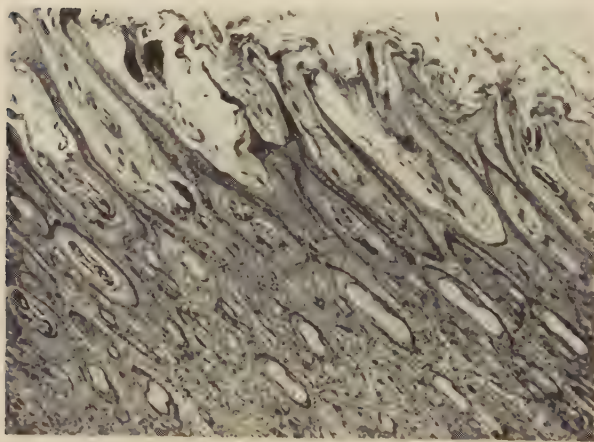


FIG. 4.—Section of skin from a rabbit of the 18th rabbit passage, eight days after scaliness and erythema were first noted and 17 days after inoculation with dried scrapings of rabbit lesions that had been stored for 267 days. There is great hyperplasia of the upper parts of the hair follicles and the dermis is congested, oedematous, and heavily infiltrated by inflammatory cells. Haematoxylin and eosin, $\times 30$.

The Experimental Disease

Lamb. A lesion from the flank of Lamb 4, removed 19 days after inoculation, shows elongation of the papillae with proliferation of the epidermis and of the epithelium of the hair follicles. In the epidermis at the tips of the papillae there are vesicles which appear to have arisen by the coalescence of locules formed by progressive vacuolation of the epithelial cells and by increasing oedema of the intercellular spaces (Fig. 3). The dermis is oedematous, congested, and heavily infiltrated by mononuclear cells and to a much less extent by polymorphonuclear leucocytes.

Goat. A lesion from Kid 1, removed 14 days after inoculation, shows changes similar to those in Lamb 4. There is, however, greater hyperplasia of the epidermis and hair sheaths, and there is no evidence of the formation of vesicles. The cellular infiltration is again heavy, consisting mainly of mononuclear cells. Some of the hyperplastic hair follicles are infected and invaded by polymorphonuclear leucocytes which, in places, extend into the surrounding tissues.

Rabbit. The specific characters of the infection in the rabbit are shown in Fig. 4 of a section of skin from a rabbit taken 17 days after inoculation and 8 days after scaliness was first noted. There is great hyperplasia of the upper parts of the hair follicles which appear to have largely displaced the epidermis, forming an almost papillomatous surface covered with abundant keratin. In the more superficial part of the dermis there is considerable congestion and oedema and a heavy infiltration by inflammatory cells. The infiltrating cells are mainly mononuclear cells but polymorphonuclear cells are also present particularly in association with infected hair follicles. Congestion and inflammatory infiltration are also present in the depths of the dermis but to a much more moderate extent.

DISCUSSION

It would appear from the published reports on contagious pustular dermatitis that, besides the sheep, the only animal that is susceptible to the virus is the goat. Numerous investigators have reported their failure to transmit this infection to the smaller laboratory animals and among those who have had no success with rabbits are Aynaud (1923), Jacotot (1926), Glover (1928), Howarth (1929), and Boughton and Hardy (1934). Lanfranchi (1925), however, claims to have successfully infected young rabbits by inoculation on the lips. He states that papules and pustules appeared five days after inoculation and that the lesions later became covered with crusts. The experiment was terminated ten days later by the rabbits dying of coccidiosis. The lesions were not examined histologically nor were they transmitted to other animals, so that there would appear to be considerable doubt whether the lesions were caused by the sheep virus.

It is already apparent that the experimental rabbit infection

described here is caused by a virus in that proliferative changes characteristic of the action of viruses were produced in the lamb and the rabbit by Berkefeld filtrates. It is also apparent that the rabbit infection was probably derived from the natural disease in Lamb 1, because lesions appeared simultaneously in all three inoculated rabbits in the first transmission with material that had already produced characteristic lesions in Lambs 4 and 5, and which, again, in a later experiment, produced a typical lesion in a rabbit in another laboratory. Furthermore, no natural immunity to the virus has been observed in laboratory rabbits so that they would not appear to harbour the virus in a latent form; and, finally, there is no record of an infection in any way similar to the experimental infection having been observed in rabbits.

The next question that has to be considered is whether the virus now established in rabbits can be identified with the virus of contagious pustular dermatitis. It has been shown that the rabbit-passaged virus was probably derived from the natural disease in Lamb 1, which, in its clinical and histological features, is characteristic of the papillomatous form of the sheep infection as described by Glover (1928). The history of the lamb being one of 19 affected lambs in a flock of 52 in a district where previous outbreaks had been observed is also typical of the disease (Glover, 1928, 1934-1935). Passage of the lesion in Lamb 1 to normal lambs and kids also resulted in an infection which, in its latent period, clinical appearance, and course was similar to Glover's experimental infection. The detailed tissue changes in Lamb 4 (Fig. 3), such as the hyperplasia of the epithelium and the formation of intra-epidermal vesicles, are also essentially similar to those figured by Glover, although the general histological picture differs somewhat because of the great hyperplasia of the hair follicles present in Lamb 4 and absent in Glover's lesions which were produced in the hairless skin of the thigh. It may therefore be concluded that the natural disease in Lamb 1 and the experimental infection in lambs were caused by the virus of sheep dermatitis.

The longer latent period and the prolonged course of the experimental disease in rabbits would at first appear to be against identifying the causal agent with the virus of contagious pustular dermatitis which in lambs usually produces lesions after a latent period of 4 to 5 days, with a total duration of 20 to 25 days. The rabbit infection, however, is very similar to the more chronic, papillomatous form of the natural infection both in its course and in the histological characters of the lesions. Further evidence in this respect has recently been obtained from a second transmission to the rabbit with dried material from an outbreak of contagious pustular dermatitis supplied by Mr. F. Blakemore, of the Institute of Animal Pathology, Cambridge. The resulting infection was in every respect similar to the rabbit-passaged disease except that re-transmission to rabbits

failed. It is likely, therefore, that the rabbit lesions described here are characteristic of infection with the virus of contagious pustular dermatitis.

Further evidence of the identity of the experimental and natural virus has been obtained by the demonstration of neutralising antibodies in the sera of lambs recovered from contagious pustular dermatitis. These and other confirmatory findings on the properties of the virus will be described in detail in a later communication.

SUMMARY

A strain of the virus of contagious pustular dermatitis of the sheep has probably been transmitted to the rabbit.

This virus strain is now in its 19th rabbit passage, after alternate periods of passage through rabbits and storage either in 50 per cent. glycerine or in the dried state.

The lesions produced by the virus in rabbits are more proliferative than those usually found in the natural disease, but are comparable to the papillomatous form of the disease, which is occasionally found on the lips of lambs.

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THE OCCURRENCE OF HAEMOLYTIC COCCO-BACILLI IN THE NOSE OF NORMAL SHEEP AND CATTLE

By

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INTRODUCTION

DURING the early part of September, 1940, an outbreak of nasal catarrh occurred in a flock of Border Leicester Cheviot ewes. In a flock of 199 there were 175 recently purchased and 24 older ewes which had been left on the farm. The outbreak was preceded by a few cold nights and within three days at least one-half of the flock showed a nasal discharge and some were coughing. Although the condition cleared up quickly without treatment, nasal swabs were taken from 51 of the affected ewes and these were grown on 5 per cent. ox blood agar plates. After 24 hours' incubation at 37° C. the predominating organism appeared to be a Gram-negative haemolytic cocco-bacillus resembling an organism which is associated with calf and sheep pneumonia. As the significance of these results was not apparent the examination was extended to include a number of normal sheep and cattle.

Bacteria similar to the haemolytic cocco-bacillus have been described by workers in America and this country. Jones (1921*a* and *b*) and Jones and Little (1921, 1922) recovered an organism from cases of pneumonia and rhinitis in calves and adult bovines in America and grouped it with the *Pasteurella*, calling it *B. bovissepticus* Group I. The strains studied by Jones (1921*a*) were Gram-negative rods, and fermented glucose, lactose, saccharose, maltose and mannite, produced clear zones of haemolysis in blood agar plates, were not soluble in bile and did not form indole. Their virulence for laboratory animals was low. All of eight strains studied were agglutinated by a serum prepared against one strain and the agglutinins could be absorbed by any one of the strains. Tweed and Edington (1930) reported on nine strains of a similar organism isolated from the pneumonic lungs of bovines in this country. Four of them were non-haemolytic, none formed indole, and all were proved by agglutination and absorption tests to belong to Group I of Jones. Lovell and Hughes (1935) recovered similar organisms from the lungs of calves dead from pneumonia. They also found the pathogenicity of the organism to be low for experimental animals and as they were not prepared to group it with the *Pasteurella* they referred to it as a "haemolytic cocco-bacillus." Similar bacteria have been isolated from the pneumonic lungs of sheep in Iceland by Dungal (1931) and in this country by Montgomerie, Bosworth and Glover (1938). Those studied by Dungal were not haemolytic and were apparently more virulent. One strain, however, was similar serologically to some of those studied by Montgomerie *et al.* whose strains were haemolytic and were divisible into two sub-groups by agglutination tests. None of their organisms appeared to be related serologically to Group I (Jones), but the strain obtained from Dungal fell into their Sub-Group II; two strains isolated by Lovell and Hughes from calves fell into their Sub-Group I.

There appears to be a collection of bacteria recoverable from cattle and sheep and associated with pneumonia in these species which, though varying somewhat in haemolytic power and antigenic structure, show a general similarity to those described by Jones as *B. bovissepticus* Group I. It has been recorded that recovered animals may carry these organisms in their upper respiratory passages for varying periods but little attention has been paid to their natural distribution although Francis (1940) recovered six strains from the tonsils of 100 bovine heads collected at slaughter and *post-mortem* houses. Five strains were studied and one was related antigenically to Sub-Group I of Montgomerie *et al.* and one to their Sub-Group II; the other three strains were antigenically distinct.

PRESENT INVESTIGATION

In addition to the nasal swabs from the 51 ewes already referred to, swabs were taken from the nasal cavities of 100 normal healthy sheep, 50 from each of two flocks, and from the lower portion of the trachea of 84 sheep shortly after slaughter. Swabs were also taken from the nose of a total of 113 cows and calves in three different herds. The swabs were inserted well into both nasal cavities, or into the trachea in the case of the slaughtered sheep, and were rubbed lightly over the surface of 5 per cent. ox blood agar plates; these were incubated at 37° C. and examined after 24 and 48 hours. Suspicious colonies were filmed and strains isolated; some were studied bio-chemically and by serological means. The general results obtained are given in the following table.

NUMBER OF STRAINS OF HAEMOLYTIC COCCO-BACILLI RECOVERED FROM GROUPS OF SHEEP AND CATTLE

<i>Number and Species of Animal</i>	<i>State of Health</i>	<i>Site of Swab</i>	<i>Number of Isolations</i>	<i>Time of Year of Examination</i>
51 sheep	Nasal catarrh	Nose	26	September
50 sheep	Healthy	Nose	26	November
50 sheep	Healthy	Nose	14	March
84 sheep	Healthy at p.m.	Lower portion of trachea	7	November
6 cattle	Healthy	Nose	0	February
49 cattle	Healthy	Nose	6	February
50 cattle	Healthy	Nose	3	March

In addition *Corynebacterium pyogenes* was recovered from one of the second batch of sheep, from six of the third batch and from two of the 84 tracheal swabs.

The bacteria were considered to belong to the group of "haemolytic cocco-bacilli" if they conformed to a general picture concerning their morphological and cultural characteristics. They were non-motile and films prepared from blood agar plates or agar slopes grown at 37° C. for 20 to 24 hours consisted of Gram-negative cocco-bacilli.

Some were short stumpy rods with rounded ends and others were definitely ovoid; a few short filaments were present in some films. Growth on agar slopes occurred readily at 37° C. after overnight incubation and individual colonies were smooth and greyish in colour. A uniform turbidity occurred in broth and there was some improvement in growth with the addition of glucose and blood. A narrow zone of haemolysis was present around colonies on ox blood agar plates with no appreciable haemolysis in blood broth cultures. A few strains tested under anaerobic conditions and in an atmosphere containing 20 per cent. CO₂ showed their ability to grow on blood agar under these conditions with a very much reduced growth on agar or in plain broth. Sugar fermentations were studied in a peptone water medium containing 10 per cent. broth with 7.5 per cent. brom-thymol-blue (B.D.H. indicator solution); the slight acidity formed is more apparent than with Andrade's indicator (Lovell and Hughes, 1935). Of 58 strains tested biochemically, 57 formed acid from glucose, 47 from maltose, 47 from mannite and 29 from lactose though with lactose acid was rarely apparent until after five or more days' incubation: of 43 strains tested against saccharose and salicin 40 fermented the former and three the latter. Five of nine strains from cattle, though in other respects similar to haemolytic cocco-bacilli, formed indole; all of 49 strains from sheep failed to form indole. Little or no change developed in litmus milk.

Sera were prepared in rabbits against two strains recovered from sheep, one strain which had been isolated from a case of calf pneumonia and two strains studied by Montgomerie *et al.* and classified as their Sub-Groups I and II. The clear-cut results obtained by these authors were not obtained by us as some cross agglutination occurred but it was possible to show the serological affinity of 36 of 55 strains examined, most of which came from normal sheep. Twenty-four appeared to fall into their Sub-Group I, one in their Sub-Group II, and eleven showed cross agglutination with sera prepared against Sub-Groups I and II. No agglutination occurred with the remaining 19 strains.

DISCUSSION

Omitting the sheep which were suffering from nasal catarrh, haemolytic cocco-bacilli were recovered from the nose of 40 of 100 healthy sheep and from the trachea of seven of 84; the figures for cattle are much lower. It would appear then that organisms of this class are commonly found in the nasal passages of sheep and the significance of their recovery from nasal catarrh and pneumonia should be assessed accordingly. It has been assumed that they are the actual cause of pneumonic conditions in calves and sheep although Montgomerie *et al.* suggest that while such organisms may be largely responsible for the severe terminal pneumonia it is highly probable that they are unable to gain an entrance until a breach has been effected by

some other cause; they consider that it has yet to be determined whether the initial stimulus is due to the action of a filtrable virus or is dependent upon some environmental change. With this view we are in agreement. As far as can be judged from this limited survey it appears as if these bacteria are commoner in the nose of sheep than in cattle. Too much attention must not be paid to the actual figures obtained since our examination was not a searching one and only one swab was taken from each animal. It is well known that the more searching the bacteriological examination and the more frequently it is made the higher the carrier rate. This aspect is emphasised in the report on a study of the nasopharyngeal flora of human beings in London and S.E. England made by Straker, Hill and Lovell (1939) during the years 1930-37. These authors included in their survey such potential pathogens as *Strept. pneumoniae*, haemolytic streptococcus, *H. influenzae* and the meningococcus. A particularly important paragraph in the prefatory note to this report reads as follows: "The figures collected in this inquiry show that, within relatively few years, most of the persons examined harboured, at one time or another, each of the bacteria under study. Had the swabbings been more frequent, or the inquiry more protracted, there can be little doubt that the proportion of carriers would, in each case, have approximated to 100 per cent." We feel that this has some application concerning the haemolytic cocco-bacillus in sheep.

SUMMARY

A brief survey has shown that haemolytic cocco-bacilli similar to those associated with pneumonia in cattle and sheep are often present in the upper respiratory tract of normal sheep and occasionally in cattle.

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VARIATIONS IN THE LEUCOCYTE COUNT OF THE PIG DURING THE FIRST TWELVE WEEKS OF LIFE

By

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INTRODUCTION

IN the course of certain investigations it was necessary to perform weekly differential blood counts on young pigs. Whilst total and differential counts for normal pigs had been recorded by various authors (see Table I) no reference was found giving the data required. It was therefore decided to study the leucocyte picture in two litters of newly born pigs to determine what variations, if any, were to be found.

TABLE I
LEUCOCYTE COUNT RECORDED BY VARIOUS AUTHORS

<i>Author</i>	<i>Age</i>	<i>Total Count</i>	<i>Neutrophils</i>	<i>Eosinophils</i>	<i>Basophils</i>	<i>Lymphocytes</i>	<i>Mono-cytes</i>
Scarborough* (1931)	?	15.8	39	4.5	1.2	52.1	3.3
Wintrobe (1942)	?	7-20					
Fraser (1938)	1 to 3 days	20	61	2.9	0.3	32.3	3.7
	7 to 28 days	10	39.6	0.8	0.1	55.7	5.5
	2 to 7 mths.	21	39.9	5.5	0.5	48.0	6.1
Giltner (1907)	2½ mths.	18	33.8	3.4	0.4	60.0	2.4

* Average of 140 counts by 12 authors.

Total Count in thousands per c.mm.

Differential Count as percentage.

METHODS

General.—The in-pig sows were brought in from pasture, where they were kept whilst pregnant, a fortnight before farrowing. During pregnancy and lactation they were fed on a ration of 60 per cent. pollards, 30 per cent. bean meal, 10 per cent. fish meal, at a rate of 6 lb. per day whilst pregnant and 10 lb. per day whilst lactating. Both sows farrowed on 2.7.43. One litter (Litter A) had access to green food whilst the other litter (Litter B) received no supplement.

Weaning was commenced at three weeks and the sows removed at six weeks. The ration used for weaning was composed of 85 per cent. pollards, 10 per cent. bean meal, 5 per cent. fish meal. The proportions of bean meal and fish meal were increased to 20 per cent. and 10 per cent. respectively at 6 weeks.

Haematological.—1.25 c.c. of a 0.2 per cent. solution of potassium oxalate was added to each of a series of small tubes which were graduated to hold 1 c.c. This solution was then evaporated to dryness. 1 c.c. of blood was collected from an ear vein into a tube treated as stated above, and two or more films were made from the fresh blood. The blood was always taken between 09.30 and 10.00 hours.

Total Count.—This was performed by means of a Neubauer Counting Chamber. The number of leucocytes in five large (1 sq. mm.) squares were counted. Two counts on two separate dilutions were made from each sample. The blood was diluted to 1 in 20 in a leucocyte pipette, using as a diluting fluid 5 per cent. acetic acid in distilled water with 0.1 per cent. methyl green added as a dye.

Differential Count.—The slides were stained with Leishmann using as a diluent a buffer solution of pH 6.8 containing 0.5 per cent. disodium hydrogen phosphate and 0.65 per cent. potassium dihydrogen phosphate (Whitby and Britton, 1942): they were mounted in a medium containing Distrene "80" 10 g., tricresyl phosphate 1.5 g., xylol 40 c.c. No. 0 coverslips were used.

The slides were examined under a one-twelfth inch oil immersion lens with a No. 5 eyepiece. Measurements were made by a Leitz eyepiece micrometer calibrated against a graduated slide. A minimum of 250 cells was counted on each slide.

DESCRIPTION OF LEUCOCYTES

POLYMORPHONUCLEARS

(a) *Neutrophil.*—Average size 11μ . The nucleus stains a deep red and possesses a coarse chromatin network. There is great variation in shape, particularly in the unilobed forms. Cells with more than three lobes are uncommon. The cytoplasm stains a faint orange pink and contains numerous fine pale pink granules.

Classification.—It has been shown by Kennedy and Climenko (1931) in their work on the Arneth Count that in adult pigs the majority of neutrophils falls into Class I of Cooke (Cooke and Ponder, 1927). Schilling's (1911) method was used in this investigation and a summary of the results obtained is shown in Table II. These support the findings of Kennedy but fail to agree with those of Fraser (1938).

TABLE II
SCHILLING HAEMOGRAM

Age	Myelocytes	Juveniles	Stab	Segmented
	Per cent.	Per cent.	Per cent.	Per cent.
1 week	2	7	72	19
4 weeks	0	6	74	20
8 weeks	0	2	73	24
12 weeks	0	2	75	23

The results recorded above are the average values for both Litter A and Litter B.

(b) *Eosinophil.*—Average size 12μ . The cytoplasm is tightly packed with coarse orange granules that do not, however, occlude the nucleus which is clearly visible and is usually eccentric in position. No nuclei of more than two lobes have been seen; a nucleus of the stab form is the predominant type. Occasionally bilobed forms have been observed.

(c) *Basophil*.—Average size 12μ . This cell is rare in the pig; only the more common type described by Fraser (1938), namely, the cell packed with coarse basophilic granules, has been observed.

LYMPHOCYTES

These vary in size from 5.5μ to 13μ . The majority of cells tends to be of two sizes, either from 7 to 8μ or from 11.5 to 12.5μ .

The *Small Lymphocytes* contain a well defined round or more rarely slightly indented nucleus which is irregular in staining containing many heavy chromatin masses. The cytoplasm is scanty and may be represented by a dark blue rim around the nucleus.

The *Large Lymphocyte*.—The nucleus of this cell is either oval or indented and stains a paler pink than the small lymphocyte. The chromatin is more evenly distributed than in any other leucocyte here mentioned. The cytoplasm is more abundant than that found in the small lymphocyte and stains a clear blue. Azurophil granules have been noted in a small percentage of these cells.

MONOCYTES

Average size 13.5μ . The nucleus shows a high degree of variability in shape whilst remaining monolobular. Round, oval, reniform, horseshoe and convoluted forms have been encountered. The chromatin is arranged in a characteristic lace-like network with thickened areas at the intersections of the strands. The cytoplasm is a cloudy grey colour. Fine reddish granules have been observed in the cytoplasm of some cells but this is not common.

RESULTS

These are set out in Tables III and IV and shown graphically in Figs. 1 and 2. To conserve space only three sets of values have been recorded for any one day, *viz.*, the average values for the litter and the maximum and minimum values for total count and each type of cell.

The graphs and tables show that the variations in counts follow the same general pattern in each litter. The differences are that in Litter A the variations are more distinct than in Litter B. There is a more rapid rise in the neutrophil count after the preliminary fall and the final level obtained by the lymphocytes is higher. In both litters there was a steady rise in the lymphocyte count until the litters were between six and seven weeks old, after which the count remained relatively constant for the remainder of the period under review.

The neutrophils showed a greater degree of variation in number. Following the preliminary fall there was a period of low values, after which the count rose. There was a slight fall subsequently which coincided with the removal of the sows. The count then rose again, reaching its peak at about the tenth week, after which there was a steady fall.

TABLE III
LITTER A. (10 PIGS)

<i>Age in Days</i>		<i>Total Count in 10³</i>	<i>Neutrophil</i>	<i>Eosinophil</i>	<i>Basophil</i>	<i>Lymphocyte</i>	<i>Mono-cyte</i>
6	Av.	7.00	4,090	67	6	2,680	160
	Min.	5.7	3,062	30	0	2,150	90
	Max.	8.15	5,196	124	29	3,091	256
11	Av.	6.33	2,867	32	0	3,310	128
	Min.	5.29	1,817	0	0	1,995	72
	Max.	7.99	4,798	86	0	4,483	210
14	Av.	6.54	1,985	64	4	4,320	178
	Min.	5.75	1,479	0	0	3,654	63
	Max.	7.57	2,204	266	34	4,914	304
19	Av.	7.57	2,569	55	0	4,767	172
	Min.	5.9	1,725	0	0	4,016	30
	Max.	8.8	4,124	208	0	6,173	268
24	Av.	7.85	2,881	45	8	4,766	150
	Min.	6.13	1,427	0	0	3,120	40
	Max.	9.69	3,836	107	40	5,696	250
32	Av.	10.99	5,150	68	9	5,522	240
	Min.	7.19	1,470	0	0	4,870	0
	Max.	24.30	16,910	152	62	6,668	440
38	Av.	15.43	6,837	146	12	8,126	308
	Min.	10.20	2,748	0	0	6,439	85
	Max.	19.17	10,540	478	96	10,410	382
45	Av.	13.58	4,998	208	9	7,992	369
	Min.	10.5	2,310	53	0	6,569	258
	Max.	18.03	9,736	323	65	9,088	569
52	Av.	17.28	8,012	418	24	8,386	435
	Min.	13.3	4,455	200	0	7,773	256
	Max.	22.76	10,810	589	116	10,700	681
59	Av.	18.95	9,950	152	0	8,346	501
	Min.	13.95	6,831	0	0	6,622	258
	Max.	24.53	15,350	380	0	12,850	738
67	Av.	20.12	10,745	313	0	8,505	559
	Min.	16.87	8,163	87	0	6,766	463
	Max.	25.17	14,280	708	0	12,900	690
76	Av.	17.63	8,573	336	0	8,267	357
	Min.	15.06	6,627	226	0	7,120	291
	Max.	20.28	10,750	644	0	9,274	435
81	Av.	16.99	7,699	445	62	8,340	448
	Min.	12.5	5,595	374	0	6,955	380
	Max.	20.28	10,100	558	199	10,540	780
84	Av.	15.98	6,806	479	0	8,372	321
	Min.	13.70	5,362	443	0	7,762	165
	Max.	18.44	7,784	502	0	9,860	520

All values are per c.mm.

TABLE IV
LITTER B. (8 PIGS)

<i>Age in Days</i>		<i>Total Count in 10³</i>	<i>Neutrophil</i>	<i>Eosinophil</i>	<i>Basophil</i>	<i>Lymphocyte</i>	<i>Mono-cyte</i>
5	Av.	8.15	5,064	54	6	2,840	184
	Min.	7.5	3,894	0	0	1,950	103
	Max.	11.15	7,990	144	48	3,082	308
7	Av.	6.89	3,502	69	12	3,172	148
	Min.	6.76	2,973	0	0	2,461	138
	Max.	6.93	3,805	173	35	3,374	168
10	Av.	6.86	2,848	30	4	3,810	165
	Min.	6.56	2,140	0	0	2,979	66
	Max.	7.65	3,045	120	30	4,589	321
14	Av.	6.92	2,614	102	13	3,978	275
	Min.	6.20	1,960	26	0	3,054	195
	Max.	7.63	2,979	288	33	4,655	382
17	Av.	5.94	1,858	64	0	3,817	200
	Min.	5.07	1,217	0	0	3,178	127
	Max.	7.25	2,500	108	0	4,833	242
21	Av.	7.63	2,023	120	16	5,224	248
	Min.	5.20	1,275	0	0	3,432	78
	Max.	9.87	2,810	242	47	6,708	320
25	Av.	7.38	1,865	138	0	5,195	186
	Min.	5.87	1,144	31	0	4,431	108
	Max.	9.5	2,997	410	0	6,395	248
28	Av.	7.38	2,190	108	14	4,870	201
	Min.	5.22	1,212	34	0	3,818	53
	Max.	11.5	5,923	158	43	5,685	345
35	Av.	7.54	1,885	123	9	5,340	186
	Min.	5.73	903	43	0	3,066	86
	Max.	9.60	2,295	244	43	6,732	301
41	Av.	11.7	4,612	219	9	6,684	221
	Min.	9.17	1,780	51	0	5,824	105
	Max.	14.80	5,769	394	14	8,704	469
49	Av.	11.85	4,278	275	0	6,707	378
	Min.	8.83	2,710	114	0	5,543	255
	Max.	13.60	5,626	638	0	8,283	540
54	Av.	15.76	8,149	278	0	6,947	436
	Min.	10.57	4,700	106	0	5,439	212
	Max.	21.6	13,190	404	0	8,726	640
61	Av.	15.67	9,514	155	0	6,606	373
	Min.	11.50	6,034	57	0	5,345	115
	Max.	23.23	14,630	444	0	7,782	581
70	Av.	16.2	8,650	290	20	6,930	403
	Min.	13.9	4,769	103	0	5.28	91
	Max.	21.4	14,030	664	112	8.49	693
77	Av.	12.24	6,817	174	0	6,943	305
	Min.	10.58	3,580	259	0	6,437	153
	Max.	15.3	8,187	130	0	7,998	455

All values are per c.mm.

GRAPHS SHOWING VARIATION IN AVERAGE VALUES OF NEUTROPHILS AND LYMPHOCYTES

LITTER A.

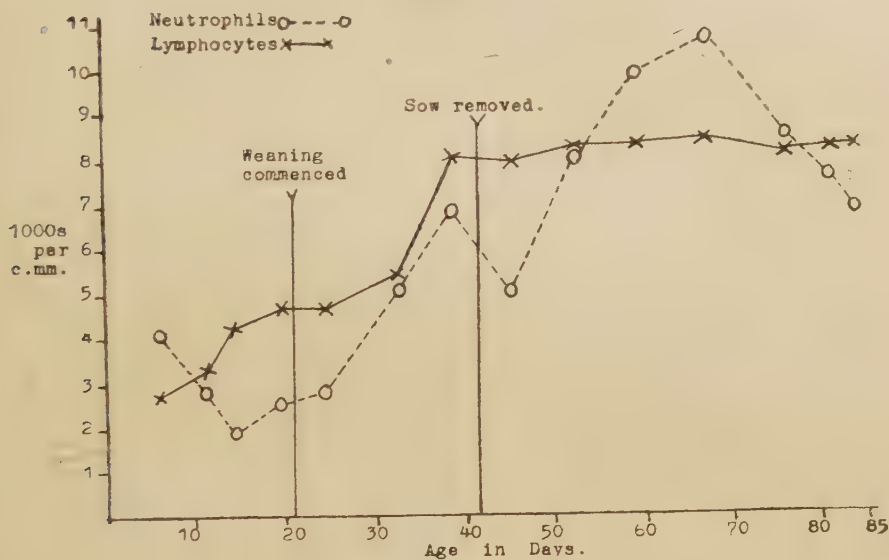


FIG. 1

LITTER B.

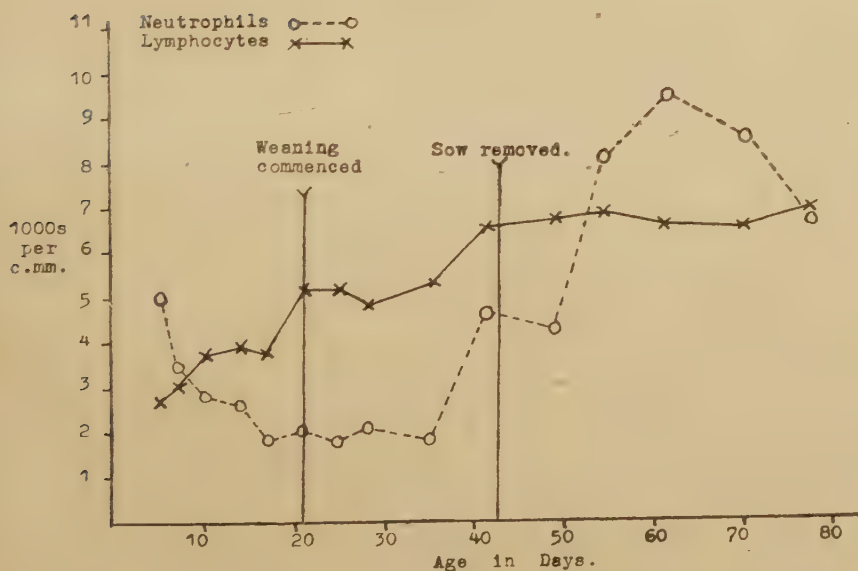


FIG. 2

The eosinophil count increased in both litters, attaining its maximum at about the seventh week.

The monocyte count increased in the period under survey; in the latter half of the period the values fluctuated, varying in Litter A between 350 and 550 per c.mm. and in litter B between 300 and 450 per c.mm.

There was no increase in basophils which remained infrequent throughout the period.

SUMMARY

A description is given of the leucocytes found in young pigs.

The variations in the leucocyte count from one to twelve weeks are recorded. Two litters were used, one of which had access to green food.

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STUDIES ON THE HAEMATOLOGY OF SHEEP

II. INDIVIDUAL DAILY VARIATION

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INTRODUCTION

THE normal ranges for the various attributes of the blood of healthy sheep, as given in the first article (Holman, 1944), are all very wide. Hence it is necessary to point out that variations occurring in serial observations on individual sheep may be significant even though they fall well within the range derived from a considerable number of animals. For example, although the normal range for haemoglobin extends from 8.6 to 15.8 g. per 100 ml., it is exceedingly unlikely for the same sheep to show a figure as high as 15 g. one day and a figure as low as 9 g. the next day. In view of this it is desirable to form some estimate of the range to be expected when observations are made on successive days on the same animal and the object of this paper is to obtain such an estimate of the expected range of the differences of such pairs of consecutive readings when no abnormal factor intervenes.

EXPERIMENTAL

To determine such a range some suitable standard of probability must be adopted—as in all similar statistical problems. No absolute upper limit can in general be set to the uncontrollable variations that may be found in one animal within 24 hours; it is necessary, therefore, to be content to find a value which, in the absence of some interfering factor, on the average will be exceeded only in some small fraction of all observations; the smaller the fraction chosen the greater this value will be. For this paper the value so calculated has been termed the “Maximum Admissible Difference,” or M.A.D., with the implied probability added in brackets. Thus M.A.D. (99 per cent.) would represent the maximum difference between two readings on the same animal that is likely to occur when no interfering factor has intervened, or, more precisely, a difference that would be exceeded only once in a hundred times in the absence of any such interfering factor. In biological experiments it has been found advisable for many purposes to take the fraction as one-twentieth (corresponding to 95 per cent.) and to consider a difference or deviation as significant when it lies beyond the limit corresponding to this fraction. This means that the limit is exceeded in untreated animals only about once in twenty observations. Any effect of less magnitude is dismissed as probably due to the normal biological variation and experimental error, whilst any greater effect is con-

sidered more or less significant according to the extent the limit is exceeded and the circumstances of the experiment.

The problem, then, is to calculate a value that is likely to be exceeded once in twenty times, at the most, by the difference between two observations made at an interval of 24 hours. There will be such a value for each blood constituent examined, and it will be dependent largely on the experimental technique used, the skill of the operator, and probably such factors as the condition and environment of the sheep. The M.A.D.s given in Table I, therefore, apply only when the technique described in the previous paper is carried out and the general conditions applying in that work are satisfied.

To calculate these M.A.D.s the method given by Mainland (1938) has been employed. In Table I (part 1), column 1 denotes the item, column 2 the number of sheep from which data are obtained, column 3 the standard deviation of the 24-hour differences in individual sheep, and column 4 the M.A.D. (95 per cent.) calculated by multiplying the standard deviation given in column 3 by the appropriate value in the table of "t" (Fisher and Yates, 1943), corresponding to a probability of one-twentieth; the use of the table of "t" for this purpose is suggested by Mainland (1938) and for this n is taken as the number of sheep given in column 2.

TABLE I

		PART 1 Daily Variation			PART 2 Monthly Variation		
		Sheep	S.D.	M.A.D. 95%	Sheep	S.D.	M.A.D. 95%
W.C. 10^3 per c.mm.	...	16	1.87	4.0	20	3.34	7.0
Band forms, %	...	16	0.47	1.0	20	1.24	2.6
Polymorphonuclears, %	...	16	7.35	15.6	20	15.53	32.4
Lymphocytes, %	...	16	7.50	16.0	20	15.13	31.6
Basophils, %	...	16	0.76	1.6	20	1.25	2.6
Monocytes, %	...	16	1.57	3.3	20	1.40	2.9
Eosinophils, %	...	16	3.66	7.8	20	3.28	6.8
R.B.C.s, 10^6 per c.mm.	...	21	1.34	2.8	20	1.70	3.6
Hb, g. per 100 ml.	...	20	0.94	1.9	14	1.88	4.0
P.C.V., %	...	21	3.24	6.8	20	2.90	6.1
M.C.V., cubic micra.	...	21	3.68	7.7	20	4.76	9.9
M.C.H.C., %	...	20	4.73	9.7	14	5.84	12.5
Frag. NaCl, g. per 10^4 ml.	...	21	1.54	3.2	20	2.74	5.7
S.G. aq. = 1,000	...	14	3.46	7.6	12	4.69	10.6
Viscosity, aq. = 1	...	19	1.17	2.4	18	1.55	3.3
Clotting time, min.	...	13	0.69	1.5	11	0.99	2.2

Table I (part 2) refers to pairs of observations made at an interval of not 24 hours, but one month. As would be expected, for most items the M.A.D.s are, here, somewhat greater but there are three exceptions, namely, eosinophils, monocytes, and the packed cell volume. It must, however, be remembered that these M.A.D.s

themselves are subject to considerable errors of sampling and of experiment, and such accidental errors may account for the monthly range being somewhat less than the daily range for these three items. In other words, the results are consistent with the monthly M.A.D. being at least equal to the daily one. It should be stated that for some of the items in Table I (part 1) the results were calculated from data that included a few sheep bled at an interval of 48 hours, but inspection showed that these figures were statistically homogeneous with the 24-hour data, and did not significantly alter the results.

Among the items in Table I the experimental error in estimation is highest in the classification of leucocytes by the differential count, and as the leucocytic picture is most important from the clinical point of view, an estimation of the experimental error alone has been made for comparison. It can be seen from the results given in Table II that while the total leucocyte count and the estimation of eosinophils differ significantly from those of the daily estimation, the M.A.D. (95 per cent.) for the monocytes is by chance larger than that for the daily variation, showing that in the case of this type of cell the technique is quite inadequate for the detection of small variations due to biological rhythm.

TABLE II
EXPERIMENTAL ERROR IN ESTIMATION OF LEUCOCYTES
Based on 20 Duplicates

	<i>S.D.</i>	<i>M.A.D. 95%</i>
W.C., 10 ³ per c.mm. ...	1.13	2.3
Neutrophils* ...	5.74	11.9
Lymphocytes ...	6.49	13.6
Basophils ...	0.74	1.5
Monocytes ...	2.40	5.0
Eosinophils ...	2.00	4.2

* Including Band Forms.

SUMMARY

A table is given as a guide to the daily (and monthly) variation in various haematological constituents of the blood of individual sheep. These tables give the "Maximum Admissible Difference" for the various constituents; the M.A.D. (95 per cent.) being such that the difference of two readings made on the same sheep at an interval of 24 hours (Part 1, Table I) or one month (Part 2, Table I) should not exceed this value in more than 5 per cent. of the cases in the absence of some interfering factor. When the M.A.D. is exceeded, the existence of some effect other than sampling and experimental errors becomes probable, provided that the technique described by Holman (1944) is strictly adhered to.

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THE AROMATIC DIAMIDINES IN THE TREATMENT OF TRYPANOSOMIASIS AND BABESIASIS

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INTRODUCTION

LOURIE and Yorke (1939, a & b) published results of experimental therapy using certain members of the aromatic diamidines series which had been prepared in the research laboratories of Messrs. May and Baker and sent for trial by Dr. Ewins. Protozoan diseases of man and animals were investigated, trypanosomiasis and babesiasis being the most important from the veterinary point of view.

T. congolense infection in cattle is of great importance in tropical Africa and still remains one of the most unsatisfactory so far as treatment is concerned. Antimony compounds had established themselves as the best form of drug treatment up till shortly before the war, when Surfen "C" (Bayer) was produced and gave much more satisfactory results. Since then the aromatic diamidine series and Phenanthridinium compound 879 have been tried.

Babesiasis of cattle, horses and dogs have also been a problem, and although chemotherapy has been more successful than in trypanosome infections none of the drugs used was really satisfactory. Trypanblue introduced by Nuttall and Hadwen (1909a; 1909b) was followed by Akiron or Acaprin and other drugs; this was a great advance but in most cases the toxic and curative doses were too near.

The diamidines tested will be discussed separately and experimental data and clinical records will be given in each case where the chemotherapeutic value of the drug has been investigated. In all cases the dihydrochloride was the salt used.

4 : 4' DIAMIDINO STILBENE (M. & B. 744)

This is the most active of the series used against *T. congolense* and as low a dose as 2.5 mg./kilo produced sterilisation of the peripheral blood in our experiments but relapses occurred which could not be permanently cleared up, whilst larger doses proved toxic (*vide* Table I).

Daubney and Hudson (1941b) investigated the chemotherapy of this compound and came to the conclusion that in the doses they used, *i.e.*, up to 20 mg./kilo it was impossible to effect a complete cure in *T. congolense* infections. Only two animals were used in the single dose experiment and both were again used in a further experiment with diphenoxypentane 21 days later. Two further animals were used for an injection of three doses of 5 mg. per kilo given at 7-day intervals. Here again a complete cure was not obtained but a premunised state was arrived at with almost a normal R.B.C. count. In babesiasis of dogs Daubney and Hudson (1941b) treated 16 clinical cases of tick fever with a dose of 1.5 mg. per kilo subcutaneously in a 1 per cent. or 1.5 per cent. solution. In most of the animals

TABLE I

TREATMENT OF *T. congolense* INFECTION IN CATTLE WITH M. & B. 744

No.	Dose per Kilo	How Given	Result
4065	2.5 mg.	Intravenously	Relapsed after 11 days
4069	2.5 "	"	" " 11 "
4800	5.0 "	Intramuscularly	Relapsed after 18 days. Died 58 days after injection—Cachexia
4897	5.0 "	"	Relapsed after 17 days. Died 39 days after injection—Jaundice-Cachexia
4210	5.0 "	Intravenously	Relapsed after 27 days
3806	7.5 "	"	" " 22 "
3817	7.5 "	"	" " 18 "
4210	10 mg.	"	" " 26 "
4212	10 "	"	Trypanosomes disappeared. Microscopical examinations and sub-inoculations always negative. Died 80 days after injection—Cachexia
4065	10 "	"	Trypanosomes disappeared. Microscopical examinations and sub-inoculations always negative. Died 34 days after injection—Cachexia
4056	5 " twice at fortnightly interval	Intramuscularly	Trypanosomes disappeared. Microscopical examinations and sub-inoculations always negative. Died 54 days after 1st injection—Cachexia
4208	5 " twice at fortnightly interval	"	Trypanosomes disappeared. Microscopical examinations and sub-inoculations always negative. Died 60 days after 1st injection—Cachexia
4069	5 " on two successive days	Intravenously	Trypanosomes disappeared. Microscopical examinations always negative. Died 7 months later
3562	5 " on two successive days	Subcutaneously	Relapsed after 19 days following 2nd injection
3570	5 " on two successive days	Intravenously	Relapsed 54 days following 2nd injection
3806	10 " twice	Fortnightly interval intravenously	Blood negative microscopically throughout. Died 13 days after 2nd injection. Well marked emaciation
3817	10 " "	Fortnightly interval intravenously	Blood negative microscopically throughout. Died 23 days after 2nd injection. Well marked emaciation
3570	10 " "	Fortnightly interval intravenously	Blood negative microscopically throughout. Died 117 days after 2nd injection. Well marked emaciation.
4210	10 " "	Fortnightly interval intravenously	Blood negative throughout. Killed <i>in extremis</i> 14 days after 2nd injection. Weight dropped from 114 to 104 lb.
4960	Total of 5 mg. per kilo	0.5 mg. on successive days 10 times intramuscularly	Blood negative throughout treatment and for 12 days following last injection. Relapsed. Eventually died 2 months later. Lost weight throughout
4761	Total of 5 mg. per kilo	0.5 mg. on successive days 10 times intramuscularly	Blood negative throughout treatment. Lost condition and died about 2 months later
4945	Total of 5 mg. per kilo	0.5 mg. on successive days 10 times intramuscularly	Blood negative during treatment. Relapsed and remained positive for 2 months. Eventually negative and slowly gaining weight
4401	1.25 mg. per kilo	One only intramuscularly	Trypanosomes disappeared. Destroyed <i>in extremis</i> 2 days later (very old trypanosomiasis case)
4467	1.25 mg. six times	Total 7.5 mg. intramuscularly	Trypanosomes disappeared. Destroyed <i>in extremis</i> 15 days later (old trypanosomiasis case)

there was a reaction which varied in severity showing symptoms of hyperaesthesia, swelling of the face and in two cases dyspnoea. There were two deaths following the treatment. They concluded that the stilbene is a useful drug in tick fever with reservations in regard to undesirable reactions. They found that in biliary fever (*B. caballi* infection) of horses the drug is unsuitable owing to the undesirable reactions following its administration.

As far as this laboratory is concerned only a few cases of *B. canis* were treated, and whilst the drug is undoubtedly effective against the infection, the alarming and very undesirable reactions following the injection of the compound led to its early abandonment in favour of a less toxic compound.

4 : 4' DIAMIDINO DIMETHYL STILBENE (M. & B. 991)

A small amount of this compound was made available for tests through the courtesy of Dr. Ewins, of Messrs. May and Baker. Yorke found it almost *four* times as active as the M. & B. 744 in *T. congolense* infection in mice and it is much more readily soluble. Carmichael and Bell (1943), in a short note, give the experimental results in 20 cattle infected with *T. congolense*. The therapeutic index was found to be very narrow and toxic symptoms were too frequently observed to justify further trials.

4 : 4' DIAMIDINO DIPHENYL ETHER (M. & B. 736) OR " PHENAMIDINE "

This compound has proved of little value in *T. congolense* infections and investigations were dropped after four animals had been treated (*vide* Table II).

TABLE II

No.	Dose—M. & B. 736			Result— <i>T. congolense</i>		
4928	10 mg.	per kilo	subcutaneously	Relapsed after 7 days		
4929	10	"	intravenously	"	"	7 "
4928	15	"	"	"	"	11 "
4929	15	"	"	"	"	11 "

In babesiasis of dogs, however, the diphenyl ether is highly specific and at the same time has the advantage of low toxicity. The dose which has proved most efficient is 10 mg./kilo given subcutaneously, but two or three times this dose produces no marked reactions. Recently the dihydroxyethanesulphonate has been produced which is very much more soluble and therefore can be injected in a smaller volume. In a preliminary report, Carmichael (1942) recorded the efficiency of this compound in tick fever in dogs when ten cases were reviewed. Since then our records show that 140 dogs have been treated in various stages of the disease and of these four relapsed

8 to 17 days after treatment. There were no deaths. The relapsed cases were retreated and did not break down again. Since the inception of M. & B. 736 tick fever is no longer the serious menace it used to be in enzootic areas where it was not uncommon to lose 50 per cent. of dogs, especially imported breeds, from this disease. There is every prospect of this compound being equally efficient in babesia infections in cattle, and Pierce (1943) reports favourably on its use in Ireland.

In horses it is very non-toxic and a dose of 3 grammes subcutaneously given to an animal weighing approximately 1,000 lb. caused no ill effects. The only case treated was an imported thoroughbred stallion with an infection of *B. caballi*, in which this compound gave excellent results and there is every reason to believe that phenamidine will in future be the drug of choice in the treatment of biliary fever in horses.

4 : 4' DIAMIDINO DIPHENOXY PROPANE (M. & B. 782)

This compound has been tried in *T. congolense* infection in cattle, and whilst it is less toxic than the stilbene the results were disappointing. The maximum tolerated dose is between 5 and 10 mg. per kilo, which was insufficient to produce a cure, although it caused a temporary sterilisation of the blood. A dose of 10 mg. per kilo proved toxic. (See Table III.)

TABLE III

No.	Dose—M. & B. 782	Result— <i>T. congolense</i>
4207	2.5 mg. per kilo subcutaneously on two successive days	Relapsed after 15 days
4476	2.5 mg. per kilo intravenously on two successive days	" " 15 "
4206	5 mg. per kilo subcutaneously	" " 13 "
4061	5 " " intravenously	" " 11 " Died 8 days later
4982	10 " " subcutaneously	Trypanosomes disappeared — but died 4 days after injection — poisoned
4933	10 " " intravenously	Trypanosomes disappeared — but died 10 days after injection — poisoned

In babesiasis in dogs, however, this drug is very useful. Carmichael and Fiennes (1941) reported on 116 unselected cases in which there were ten relapses and four deaths. It is rather more toxic than M. & B. 736, the dose being 5 mg. per kilo body weight divided into two doses given on successive days. It has now been superseded by the former compound.

4 : 4' DIAMIDINO DIPHENOXY PENTANE (M. & B. 800)
" PENTAMIDINE "

This compound proved as active against *T. congolense* in mice as the Stilbene (Lourie and Yorke, 1939a) but in cattle 10 mg. and 15 mg. per kilo either failed to produce a cure or proved too toxic. It was equally disappointing in two cases of *T. vivax* infection in so far that complete sterilisation of the blood was not effected. (See Table IV.)

TABLE IV

No.	Dose—M. & B. 800	Result— <i>T. congolense</i>
3562	10 mg. per kilo intravenously	Died during injection
4930	10 " " "	Relapsed after 16 days
3945	10 " " "	Relapsed after 19 days. Eventually destroyed in very poor condition
4932	10 " " "	Trypanosomes disappeared — killed in <i>extremis</i> 29 days after injection — poor condition ; loss of weight
4928	15 " " "	Relapsed after 14 days and died 6 days later
4929	15 " " subcutaneously	Trypanosomes disappeared — killed in <i>extremis</i> 4 days after injection
No.	Dose—M. & B. 800	Result— <i>T. vivax</i>
3949	10 mg. per kilo intravenously	Relapsed after 16 days
4059	10 " " "	" " 11 "

Daubney and Hudson (1941a) investigated its trypanocidal properties against *T. congolense* in cattle and came to much the same conclusion. They also point out that there is a delayed toxic effect with fatty changes in the liver. This was also seen in two of our cases which died 19 and 29 days following treatment.

The prophylactic value of Pentamidine has been investigated in this laboratory and experiments have shown that a dose of 5 mg./kilo will protect an animal from infection with *T. congolense* for 24 hours but not for 48 hours or longer.

DISCUSSION

As far as veterinary work is concerned the aromatic diamidines have not proved to be efficient in the treatment of trypanosomiasis, especially that caused by *T. congolense*, which is by far the most important trypanosome causing disease in tropical Africa. In babesiasis of dogs, and it is likely that this will apply to babesiasis in other animals, Phenamidine (4 : 4' diamidino diphenyl ether) has proved a most valuable agent owing to its curative value combined with its very low toxicity. It is definitely a big advance on all other preparations used in the treatment of this disease.

CONCLUSIONS

The uses of the aromatic diamidines in veterinary practice are discussed, and whilst in trypanosomiasis they have proved of little practical value this series has given us a most valuable compound in Phenamidine for the treatment of babesiasis in animals. The results in 140 cases in dogs with only four relapses and no deaths are recorded.

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